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# PHYSIOLOGICAL STUDIES ON THE PRODUCTIVITY OF *GRACILARIA*

A thesis submitted to
Madurai Kamaraj University
for the degree of
**Doctor of Philosophy**

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*Dedicated*

*to*

*my beloved*

*children*

***Rajju and Sonu***

## DECLARATION

I do hereby declare that this work has been orginally carried out by me under the guidance and supervision of **Dr. G. Kulandaivelu**, Professor and Head, Department of Plant Sciences, Madurai Kamaraj University, Madurai and this work has not been submitted elsewhere for any other degree.

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## CERTIFICATE

This is to certify that this thesis entitled **"Physiological studies on the productivity of *Gracilaria*"** submitted to Madurai Kamaraj University by **Reeta Jayasankar**, Scientist, Regional Centre of CMFRI, Mandapam Camp, for the Degree of Doctor of Philosophy is based on the results of studies carried out by her under my guidance and supervision. This thesis, or any part thereof, has not been submitted elsewhere for any other degree.

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## ABBREVIATIONS

| | |
|---|---|
| ATP | Adenosine triphosphate |
| APC | Allophycocyanin |
| $BF_3$ | Barium trifluoride |
| BL | Blue light |
| BSA | Bovine serum albumin |
| Chl a | Chlorophyll a |
| Chl b | Chlorophyll b |
| DAT | Days after treatment |
| DW | Dry weight |
| FAME | Fatty acid methyl ester |
| Fm | Maximum fluorescence level |
| Fv | Variable fluorescence |
| Fo | Fluorescence at O level |
| Fw | Fresh weight |
| GC | Gas chromatography |
| GL | Green light |
| HL | High light |
| IL | Intermediate light |

| | |
|---|---|
| IL | Intermediate light |
| LL | Low light |
| $m^{-2}$ | Meter square |
| mM | Milimolar |
| µE | Micro Einestine |
| NADPH | Nicotinamide adenine dinucleotide |
| PAR | Photosynthetic active radiation |
| PFD | Photon flux density |
| PSU | Photosynthetic unit |
| PSI | Photosystem I |
| PSII | Photosystem II |
| PBP | Phycobiliprotein |
| PC | Phycocyanin |
| PE | Phycoerythrin |
| ($\phi_m$) | Quantum yield |
| RL | Red light |
| RUBP | Ribulose 1,5-biphosphate |
| Rubisco | Ribulose 1,5-biphosphate carboxylase oxygenase |
| WL | White light |

# CONTENTS

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# Introduction

About 72% of the earth's surface is covered by ocean, which can be linked to a number of basins or shallow dishes. The lip of the basin or dish corresponds to the continental shelf and main part is the ocean proper. The average depth is greater than 5000 m.

Indian coast line stretches to about 7152 Km in its South, South-east and South-west by the seas, Indian Ocean, Bay of Bengal and Arabian Sea. The rocky shores of Gujarat and Maharastra in the West and Andhra Pradesh and Tamil Nadu on the East and coral reefs of the islands off South Indian coast support a rich and varied vegetation of marine algae. The distribution of seaweeds are more prominent in the following coasts:

1. Okha port and Dwarka along Gujarat coast
2. Karwar along West coast
3. Madras Kovalam, Mahabalipuram, Tuticorin and Cape Comerin along Tamil Nadu coast
4. The islands of Gulf of Mannar, viz., Pamban and Rameswaram and a group of coral islands namely Krusadai, Shingle, Pallivasal and Hare islands
5. Visakhapatnam coast in Andhra Pradesh
6. Sundarbans of West Bengal,
7. Andaman Nicobar islands in the east and Lakshadweep in the west coast of India.

About 700 species of marine algae have been reported along the east and west coasts of India, Lakshadweep and Andaman & Nicobar islands (Kaliaperumal and Kalimuthu 1993).

The distribution of marine plants is limited to the upper edge of the ocean because of light requirements for both benthic (attached) and planktonic species. Thus, benthic or bottom-dwelling forms are found in the shallower areas of the continental shelves (to about 150 m) and free floating forms are found in upper limited areas of ocean water (to about 200 m). Marine plants are primary producers of the oceans and thus form the standing crop (biomass) and determine the productivity for all communities. Several marine plants serve as a habitat for economically important animals, although others act as litter and stabilizer of ocean water and sediment.

Seaweeds are macroscopic marine algae of the classes chlorophyceae, Phaeophyceae and Rhodophyceae. The grouping and separation of algae based on coloration have been used for over 100 years (Harvey 1841) and the modern classification of the algae still uses pigment composition as the primary character. The green algae (including Chlorophyta, Charo- phyta and Euglenophyta) have a pigmentation similar to the higher plants and contain chlorophyll a, b and β-carotene. All the remaining group of algae, contain chlorophyll a and β-carotene but lack chlorophyll b. Phaeophyta, Chrysophyta, Pyrrophyta and Cryptophyta contain chlorophyll c, but the characterstic brown color is formed from a mixture of xanthophyll. Phycobilins are the major accessory pigments of red and blue green algae.

Rhodophyta appears to have 4100 species in 675 genera. Gracilariaceae is a family under Gigartinales having six genera and 130 species, of which

100 or so belong to world wide agarophyte *Gracilaria*. Major regional studies of *Gracilaria* include those of Yamamoto (1978) for Japan, Chang and Xia (1976) for China, Umamaheswar Rao (1972) for India and Kim (1970) for Chile.

In India so far 13 species of *Gracilaria* are recorded.

1. *G. bursa - Pastoris* (Gmel) Silva
2. *G. corticata* J. Ag
3. *G. crassa* Harvey
4. *G. debilis* (Forssk) Boergs
5. *G. disticha* J. Ag.
6. *G. dura* (Ag) j. Ag.
7. *G. edulis* (Gmel) Silva
8. *G. fergusonii* J. Ag
9. *G. foliifera* (Forssk) Boergs
10. *G. obtusa* Grev
11. *G. pygmaea* Boergs
12. *G. texori* (Sur) J. Ag.
13. *G. Verrucosa* (Huds.) Papenfuss

*Gracilaria* is seldom found in areas of extreme exposure. Attached and free floating plants may occur in the same habitat, although in general the density of attached plant are seldom low. More commonly the plants are attached to shells, small stones, pebbles or small objects often with basal portion of the plant burried (Bird *et al.* 1977b, Mayer 1981, Doty and Santos 1983).

From the polysaccharide composition point of view, agar content is of primary consideration in *Gracilaria*. It is also used as a vegetable. In Tamil Nadu it is locally called as "kanji pasi'. The quality of agar is species-specific. Present estimates indicate that about 5000 tonnes of agar are processed from *Gracilaria* annually which in turn requires 25,000 to 30,000 tonnes of dry resources (McLachlan and Bird 1983). Much of world's agar is processed in Japan but the resources are obtained else where. Asia is the leading region of seaweed supply for the manufacturers of agar (Doshi *et al.* 1992). The current international market price for the raw materials of agarophytes for the manufacture of agar range from US $ 1,500 - 1,600/ton for *Gelidium* and *Gelidiella* species and US $ 1,200-1,300 ton for *Gracilaria* species.

In India, the main natural agarophyte resources consist of different species of *Gelidiella* and *Gracilaria* found in the South east coast in Mandapam-Tuticorin area of Gulf of Mannar, Pulicat lake, Andhra Pradesh coast, Chilka lake and Andaman & Nicobar islands. Patches of *Gracilaria* species are found in Arabian sea near Kanyakumari of Kerala coast and Lakshadweep islands. The area between Keelakarai and Rameswaram in South east coast of Tamil nadu of India forms the nodal region for commercial exploitation of economic

seaweeds for the indigenous phycolloid industry. Seaweed resources of Tamilnadu have extensively been investigated (Umamaheswar Rao 1969, 1972 and Kaliaperumal and Pandian 1984).

The current market price for *Gelidiella acerosa* ranges from Rs. 8000 to 10,000/ton and for *Gracilaria* spp. Rs. 3,500 to 4,500/ton. The agar production in country is around 60 tonnes per annum from a few manufacturers either in stripes and powder form. The major consumers are food industries.

Increasing demand for agarophytes coupled with depletion of natural stocks necessitates propagation of some species of *Gracilaria*. Knowledge on eco-physiological status of cultivable seaweeds is of paramount importance to determine the suitable period and optimum conditions for their growth and productivity.

The world wide primary production of benthic seaweeds may account for about 10% of that of total phytopopulation, although it occupies less then 0.1% of the area available to phytoplankton communities (Kremer 1981a). Therefore, it is evident that macroalgae contribute significantly to the productivity of marine ecosystem.

The productivity of macrophytes depends on the environmental factors and 'yield' is the cumulative effect of production which depends on substrate as well as different plant characters, such as fresh weight, dry weight, organic dry weight, chlorophyll and other pigments, biochemical and mineral constitutents. These factors may vary independently of each other

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under different conditions.

Studies on photosynthesis of benthic macroalgae by measurement of oxygen production or carbon fixation have provided a good deal of information on the production rate. The aquatic plants in confronted with conditions very different from their terrestrial counterparts (Dring 1982). The photosynthetic rate of aquatic plants are generally low (Raven *et al.* 1985) because the diffusion resistance of free $CO_2$ is $10^4$ folds greater in water than in air.

Successful estabilshment of *Gracilaria* maricultures entails use of strains giving higher and better agar yields. Many factors can influence the quality and quantity of agar during growth of seaweeds (Cote and Hanisak 1986), which include species and strain differences, polysaccharide content, distribution and growth rate, growth conditions such as nutrient levels, light and temperature, as well as age and morphology of the plants (Whyte and Englar 1980, Craiage and Wen 1984).

Species of *Gracilaria* in India hold great potential for mariculture. The present study was carried out with a view to establishing a sound database on eco-physiological status of *G. edulis, G. corticata* and *G. crassa* collected from the Gulf of Mannar coast of Tamil Nadu.

# Review of literature

The red algae comprise the largest proportion of macroscopic seaweeds. Most members are marine with only 3 fresh water which are found in the streams. A few unicelluar forms occur in the soil. The marine forms occur in a great variety of habitat from the intertidal zone to deep water.

**Systematics**

At the highest taxonomic level there is a mild dispute over, whether the red algae consists of two classes namely Florideo-phycidae and Bangiophycidae or a single class, Rhodophyceae. The former is assayed by Dixon (1973) followed by Abbott and Hollenberg (1976) and Parke and Dixon (1976) while the latter is adopted by Kylon (1956) and others (Cole and Conway 1975, Bold and Wynne 1978 and Kraft and Woelkerling 1981). The taxonomic interpretation of the genus *Gracilaria* has been highly divergent (Chang and Xia 1963, 1976, Yamamoto 1978, 1984; Abbott 1980, 1983; Bird and McLachlan 1982, 1984; Sotomayor and Almodovar 1982, De Oliveira 1984; Trono *et al.* 1983, Zhang and Xia 1984; Abbott and Norris 1985; Fredericq and Norris 1985; Umamaheswar Rao 1972). Umamaheswar Rao (1972) made a detailed study on Indian *Gracilariaceae*.

**Gracilaria** belongs to

Class - Rhodophyta

Subclass - Florideophyceae

Order - Gigartinales

Family - Gracilariaceae

Genus - Gracilaria

## Distribution

The genus *Gracilaria* is widely distributed around the world. It occupies a variety of habitats both in tropical and temperate latitudes, forming monospecific strands or multi-specific vegetational assemblage. In temperate latitudes, some species can reach high-standing stocks (1-7 $Kg.m^{-2}$) as described for the northern Adriatic sea, Norway (Stokke 1957, Simonetti *et al.* 1970) the Atlantic coast of Canada (Bird *et al.* 1977a, b; Goldstein 1981), the east coast of United States (Humm 1944, Conover 1958; Kim and Haumm 1965), California (Abbott 1980, Hansen 1984), Chile (Santelices and Fonck 1979; Westermeier 1981), Argentina (Mayer 1981, Cerezo 1986); New Zealand (Luxton 1981) and South Africa (Issac and Molteno 1952). In tropical latitudes, *Gracilaria* standing stock values do not usually exceed 2 $Kg.m^{-2}$ (Lawson 1954, Raju and Thomas 1971, Hoyle 1978, Hay and Norris 1984), although local specific diversity is normally higher than in temperate areas and the species often have sympatric patterns of distribution (Sotomayor and Almodovar 1982, Hay and Norris 1984).

## Chloroplast structure

Fine structural aspects of the chloroplast in addition to pigment composition and other biochemical criteria are of considerable significance as defining features of Rhodophyta (Dixon 1973, Duckett and Peel 1978). Chloroplasts are surrounded by an envelope composed of two membranes (10-12 nm). The chloroplast membrane encloses the matrix or stroma in which are embedded flattened membrane bound sacs called thylakoids (or photosynthetic lamellae). One or more peripheral thylakoids (or inner limiting discs, Bouck 1962) may encircle the plastid (Bisalputra 1974, Hara and Chihara 1974). The water soluble

accessory pigments, allophycocyanin, Phycocyanin and Phycoerythrin are assembled into structures known as phycobilisomes which are distributed in a distinct pattern on the thylakoid surface (Gantt and Conti 1965, Neushul 1970).

Genophores or electron transparent regions presumed to contain DNA, are typically scattered in the stroma. The DNA fibrils are attached to the thylakoid membrane which may be involved in their replication and segregation during chloroplast division (Bisalputra and Bisalputra 1967, Bisalputra 1974) Small lipid granules or plastoglobuli are also observed in the stroma (Bisalputra 1974). Floridean starch is not found within red algal chloroplast but accumulates within the cytoplasm (Borowitzka 1978, Pueshel 1979).

**Cell wall and matrix**

Electron micrographs of red algal cell wall reveal layered fibrillar appearance, though the general orientation of the fibrillar component varies considerably in different areas of the same plant as well as between different plants (Gordon and McCandless 1973, Duckett and Peel 1978). The randomly oriented microfibrils of the inner layer are composed of cellulose in Florideophyceae (Myers *et al.* 1956) and mostly xylans in Bangiophyceae (Frei and Preston 1964). The algal matrix and outer mucilagenous layers are generally composed of sulphated polysacharides (Mackie and Preston 1974).

**Productivity**

The majority of the work for last 15 years has been concerned with the seasonal productivity rates of various algae (Johnston 1969, Littler and Murray 1974, Buesa 1977, Dawes *et al.* 1978, Wallentirius 1978). Others have dealt with the strategy for growth and effects of morphology on photosynthetic performance (Mann 1973, Keith and Murray 1980, Littler 1980). The above studies led to the recent recognition of the role of seaweed communities in

marine productivity (Mann *et al.* 1980) and as a global carbon sink (Smith 1981). Changes in dissolved oxygen in light and dark bottles are used extensively to determine the photosynthesis of marine phytoplanktons and also applied in marine phytobenthos (Johnston 1969, Burkholder and Almodovar 1973, Buesa 1977, Littler 1980). Johnston and Cook (1968) demonstrated in comparative assay that oxygen method is as reliable as $^{14}C$ technique. Moreover the rapidity of measurements and improved accuracy introduced by the modern oxygen electrode and fluorescence kinetics make this method a valuable tool for investigation. Fluorescence induction of *in vivo* Chl *a* is to determine the physiological condition of the algae and basic mechanism of photosynthesis of plant (Papageorgiou 1975, Krause and Weis 1984, 1991, Renger and Schreiber 1986, Govindjee *et al.* 1986, Buchel and Wilhelm 1993). However, the majority of the macroalgal studies are handicapped by broad range of variability within experimental replicates which often exceed the seasonal and other parameters under investigation (Littler and Arnold 1980).

**Factors affecting productivity**

Among the major environmental factors affecting seaweeds and their productivity are light, temperature, salinity, water and nutrient availability.

**Light**

Seaweeds grow in an exceptionally diverse light environment and light provides the initial energy of photosynthesis and ultimately for all biological processes. In the sea, light is attenuated due to absorption and scattering. Sea salts have little influence (Morel 1974) and so the absorption of light in pure seawater is minimal in blue and maximal in red region. In coastal seawater, shorter wavelengths are absorbed by dissolved organic compounds (Craige and McLachlan 1964, Siebarth and Jensen 1968). As the solar energy penetrate the oceans it is altered in both quality and quantity. The level of irradiance needed to saturate a species shows some correlation with its habitat. Intertidal

species require 400-700 $\mu Em^{-2}s^{-1}$, upper and mid sublittoral species saturate with 150-250 $\mu Em^{-2}s^{-1}$ and deep sublittoral species require less than 100 $\mu Em^{-2}s^{-1}$ (Luning 1981).

**Chromatic adaptation and pigment composition**

A chromatic adaptation in the photosynthetic quantum yield for Chl *a* absorbed light was first found by Yocum (1951) and Blinks (1954), with red alga *Porphyra percifera* and confirmed by Brody and Emerson (1959a, b) for *Porphyridium cruentum*. Cells grown under blue and red light, which are absorbed mainly by Chl *a* show higher quantum yield than cells grown under green light which is absorbed by PE. The ratio of PBP/Chl a is higher under blue or red light and lower under green light (Brody and Emerson 1959a). Similar changes were found for cells of *Anacystis nidulans* grown under white and far red light (Jones and Myers 1965). Ley and Butler (1980) reexamined this phenomenon with *Porphyridium cruentum* and found that the Chl a form also changed. Meyers *et al.* (1978, 1980) reported that growth of *Anacystis* under far red light induced not only pigment changes but also changes in photosystem compositions; the PSI/II ratio become larger than I under the light mainly absorbed by PBP but smaller than I under far red light. Kawamura *et al.* (1979) found that the photosystem composition in blue-green algae varies with light intensity for algal growth. The RCI become larger than RCII under light limiting conditions but become equal to it under light saturating conditions. Fujita *et al.* (1985) indicated that PSII population in a cell basis is fixed but the PS1 population varies depending on chromatic environment.

Dring (1981) indicated that classical complementary chromatic adaptation observed in macroalgae (Engelmann 1883), does not appear to be a response to zonation or tidally influenced changes in light quality. He proposed that changes in pigment composition in benthic marine algae, with increasing depth, are largely adaptation to low irradiance and not spectral compositions. In

microalgae, the effect of both quality and intensity on photosynthesis and pigment composition have been illustrated (Jeffery 1984, Humbeck *et al.* 1984). Similarly the effect of light qualities on pigment composition have also been reported in macroalgae (Lopez-Figueuroa and Niell 1989a). However the controversy between intensity adaptation and chromatic adaptation has still not been resolved.

**Light and growth**

Light plays a very important role as a factor controlling plant morphology (Dring and Luning 1983, Dring 1988). Most reports analyse the effects of light quality on growth, morphology and pigmentation (Mathieson and Burns 1975, Dring 1988). The relationship between pigmentation and growth in different light qualities is not well understood and very little information is available for macroalgae (Figueroa *et al.* 1995). Long term cultivations in either blue or red light produced adverse effects in a few green and brown algae (Clauss 1970, Schmid 1984, Wennicke and Schmid 1987). For red seaweeds little is known of long term spectral effects on growth, performance or metabolism. Recently Leukart and Luning (1994) demonstrated that green light at very low intensity (0.5 $\mu$mol $m^{-2}s^{-1}$) was more effective than red or blue light for germling growth in several red algae cultivated for at least 15 weeks. The better growth rate in red than in blue light of *Porphyra umbilicalis* was probably due to high photosynthetic efficiency and quantum yield in red light (Figueroa *et al.* 1995). The light absorption around the maximal transmission of the blue light (440-480 nm) was 1.2 to 1.5 times smaller than that of maximal transmission of red light used (630-670 nm) (Figueroa *et al.* 1994). According to the model (Gantt 1990) it is expected that the external part of phycobilisomes would increase proportionally more in blue than in red light. The low photosynthetic efficiency of red algae in blue light has been well known from

the photosynthetic action spectrum (Haxo and Blinks 1950, Luning and Dring 1985). Figueroa *et al.* (1994) showed that oxygen production was three times higher in algae grown for 3 weeks in red light than algae grown in blue light at 70 $\mu$mol $m^{-2}s^{-1}$. Photoinhibition is a reversible reduction in quantum yield of photosynthesis, related to the absorption of excess light energy in which only a part can be used in photosynthesis and it ultimately produces chlorophyll bleaching (Osmond 1981). Any environmental factor which reduces photosynthesis may result in damage to photosynthetic apparatus (Powles 1984, Greer *et al.* 1986). Both low and high temperature enhanced photoinhibition have been observed in unicellular algae and higher plants (Sadakane *et al.* 1981, Ludlow 1987, Oquist *et al.* 1987).

**Temperature**

Temperature acclimation of photosynthesis and respiration have been observed in several marine algae (Rietema and Van den Hoek 1984, Davison 1987, Davison and Davison 1987). There are also numerous reports of seasonal changes in photosynthesis and respiratory metabolism (Newell and Pye 1968, Niemeck and Mathieson 1978, Yamada *et al.* 1979, Kuppers and Weidner 1980) which are consistent with temprature acclimation and suggest that acclimation is a wide spread phenonmenon in nature. Kuebler *et al.* (1991) observed gross photosynthetic responses to temperature for *Lomentaria baileyana* and *Lomentaria orcadensis*. He suggested that disruption of energy transfer between PE and Chl occur at 30°C in *L. orcadensis*. Thermal instability of thylakoid membrane has been associated with inhibition of photosynthesis in higher plants (Berry and Raison 1981) but has not previously been studied in algae where the light harvesting complexes (phycobilisoms) are

arranged on the surface of the thylakoid membrane rather than embedded within it (Gantt 1981). There is evidence that energy is preferentially transferred from the phycobilisomes to PSII (Kursur and Alberte 1983). Thus the situation in *Lomentaria* is analogous to that in higher plants where PSII is more susceptible to high temperature breakdown than PSI and produces an increase in *in vivo* chlorophyll fluorescence (Berry and Bjorkman 1980, Berry and Raison 1981).

Ocean surface temperature changes in two broad ways. They decrease towards higher latitudes from about 28°C in tropics to 0°C towards the poles. The principal environmental feature of the intertidal zone is regular exposure to atmospheric condition, so that the temperature regime is more complex than that in the subtidal region. Species from warm water area show maximum growth and presumably production between 25 and 30°C. In shallow water, production may be reduced or ceased with increasing temperature (Li *et al.* 1984, Wang *et al.* 1984). In temperate region there are marked seasonal fluctuations in water temperature in some habitats. The production is limited to a relatively short period of the year (Bird *et al.* 1977a,b). The effect of temperature on enzyme activities and the other physiological studies were illustrated by Kuppers and Weidner (1980) on *Laminaria hyperborea.*

**Salinity**

The salinity of open surface water is generally 34 to 37‰lower than those areas with great rainfall (north westcoast of North America) and higher in subtropical areas of high evaporation and low rain fall (Groen 1980). During exposure to the atmosphere, seaweeds on open rock surfaces and in tide pools may be subjected to frequent salinity fluctuations. Photosynthesis, respiration and growth tend to be favourable under optimum salinity. Lower salinity often stunt the growth of seaweed and produce variable effect on branching (Norton *et al.* 1981). At cellular level, Reed *et al.* (1980a,b) have noted that cell

division of *Porphyra porpurea* is inhibited in concentrated seawater. Intertidal seaweeds are generally able to tolerate seawater from 10-100‰subtidal algae are less tolerant, especially to increased salinities, withstanding generally 18-52‰(Biebl 1962, Gessner and Schramm 1971).

Salinity is an important environmental parameter (Gessner and Schramm 1971) affecting distribution, growth, morphology and chemical composition of algae (Haug and Larson 1958, Kim 1970). There are relatively few studies on the effect of salinity on the morphology and anatomy of algae (Levring 1969, Jordan and Vadas 1972).

The short and long term effects of salinity on the physiology of intertidal algae have been examined primarily in terms of their physiological accomodation to this stress (Munda and Kremer 1977, Bisson and Kirst 1979, Kauss 1979, Kirst and Bisson 1979, Kremer 1979a, Reed *et al.* 1980a, 1980b, 1980c, Kirst 1981, Coudret *et al.* 1983). In red algae, altered salinity results in changes in turgor. In *Porphyra purpurea*, turgor increases with decreasing salinity, while volume decreases as salinity increases in a passively regulated system (Reed *et al.* 1980b). Photosynthesis and respiration have also been shown to be affected by salinity changes (Kremer 1979b, Yarish *et al.* 1979, Kirst 1981 and Coudret *et al.* 1983 ).

**Carbon limitation**

In estimating productivity it is difficult to separate a deficiency of light from a deficiency of carbon, the ambient concentration of which is usually low (Bidwell and Mclachlan 1985). As much as four times the mass of carbon may be required for production of unit algal biomass (Pirt 1984) and with *Gracilaria* around 50% of the dry weight may be cell wall polysaccharides. In areas where there is large standing stock of *Gracilaria* it is easy to suggest that carbon may be limiting.

In submerged aquatic macrophytes, the relationship between net photosynthesis and carbon concentration in water often follows a less gradual pattern than anticipated assuming simple Michaelis-Menten kinetics. At low concentration of inorganic carbon, photosynthesis is restricted by slow rate of diffusion from the bulk medium to the site of carboxylation.

Compared with terrestrial environment, water contains a slightly lower $CO_2$ concentration at equilibrium with air (about 13 μM at 20°C). On the other hand, at pH values above 6.3, $HCO_3^-$ concentration become progressively higher than those of $CO_2$. At pH 8.2 the equilibrium concentration of $HCO_3^-$ is about 2100 μM while that of $CO_3^-$ is about 400 μM. Higher concentration of $HCO_3^-$ could provide an important inorganic carbon (Ci) source for photosynthesis in marine plants. It is recognized that marine plants can utilize $HCO_3^-$ (Bidwell and McLachlan 1985, Kerby and Raven 1985, Reiskind *et al.* 1989). Transport of inorganic carbon into the cells has been shown in a few species of marine algae (Cook *et al.* 1986, Beer and Israel 1990, Maberly 1990, Israel and Beer 1992). It is also explained that $HCO_3^-$ may be hydrated to form $CO_2$ at low extracellular pH which catalyze the activity of carbonic anhydrase (Giordano and Maberly 1989). The common marine red alga *Gracilaria conferta* is a $C_3$ plant, but features $C_4$-like photosynthetic gas exchange traits such as $O_2$ insenstivie photosynthetic rates under ambient conditions (Israel *et al.* 1991) was observed.

**Nutrients**

Sustained production requires relatively large amount of nutrients availability within the system over long periods. In semitropical and tropical regions, availability of inorganic nutrients has been implicated as the most important factor limiting seaweed productivity. At least 56 elements have been reported to be present in seaweeds (Vinogradov 1953). Plant macronutrients, C,H,O,P,K, N,S, Ca and Mg, occur in relatively high amounts in seaweeds and are utilized

directly or indirectly for cellular building blocks (O'Kelley 1974, De Boer 1981). Healey (1973) noted three general responses of algae to nutrient deficiency, reduction in phycoerythrin and other pigment content, accumulation of C-storage compounds and decrease in protein and nucleic acid content. A decrease in phycoerythrin content was observed in *Pterocladia capillacea* under N-limiting conditions (Calabrese and Felicini 1970, Neish and Shacklock 1971). Neish *et al.* (1977) demonstrated that *Chondrus crispus* cultured in N-unenriched medium had a higher carrageenan content than those grown in N-enriched medium. Agar content in *Gracilaria foliifera* was highest in plants receiving N-unenriched seawater and decreased substantially in plants receiving nitrogen enrichment (De Boer 1979).

**Tidal effect and desiccation**

Intertidal macroalgae by definition, alternate between periods of immersion in water and exposure to air with the rise and fall of the tides. There is growing evidence that many intertidal macroalgae are photosynthetically active during exposure to air. In some cases photosynthetic rates in air surpass those in water under the same temperature and light conditions (Johnson *et al.* 1974, Quadir *et al.* 1979, Oates and Murray 1983, Bidwell and Maclachlan 1985, Oates 1985, 1986, Madsen and Maberly 1990). Sometimes the macroalgae living in intertidal zone will undergo dessication. Net photosynthesis in some algae is very sensitive to the effect of recurrent drying (Wilkene *et al.* 1978, Bewley 1979, Oates and Murray 1983).

Indeed, there is a correlation between the upper vertical limit of macroalgae and their ability to tolerate emersed conditions (Mathieson and Burns 1971, Schonbeck and Norton 1979, 1980, Hodgson 1980, Dring and Brown 1982, Smith and Berry 1986, Brown 1987). The studies of Maberly and Madsen (1990) on *Fucus spiralis* and of Oates (1985, 1986) on the saccate algae,

*Colpomenia peregrina* and *Halosaccion americanum*, suggest that 30-86% of the total daily carbon fixation typically occurs under emersed conditions. The desiccation tolerance of a species can be defined by its critical water content (Luning *et al.* 1990). Desiccation beyond this limit cause irreversible damage and thus long term recovery is affected (Dring and Brown 1982). Extreme desiccation tolerance has also been observed in *Endocladia muricata* (Britting 1992) and *Fucus spiralis* (Dring and Brown 1982).

A rapid recovery of photosynthesis upon reimmersion in water, regardless of the level of prior desiccation in *Mastocarpus papillatus* was observed (Bell 1993). Rapid recovery has also been observed in two other intertidal macroalgae, *Fucus spiralis* (Madsen and Maberly 1990) and *Endocladia muricata* (Britting 1992). However, the relative importance of photosynthesis and dark respiration in air decreases as the thalli dry out. Thus, carbon gain during exposure to air can only be important if the thalli are able to remain hydrated for a substantial period of time as has been shown for *Fucus spiralis* (Maberly and Madsen 1990) and *Ulva* sp. (Beer and Eshel 1983). The saccate algae *Colpomenia peregrina* and *Halosaccion americanum* are able to retain water in an internal cavity and therby avoid desiccation and fix substantial amount of carbon while exposed to air (Oates 1985, 1986).

**Photosynthesis**

Photosynthesis is by far the largest component of primary production (Littler *et al.* 1979, Heine 1983, Levitt and Bolton 1990) and photosynthetic rates of macroalgae have been shown to be strongly affected by seasonal changes (King and Schramm 1976b, Littler *et al.* 1979). Primary productivity (dry weight produced per unit area per unit time) in aquatic habitat depends on the interaction of physical, chemical and biological factors that constrain the rate and extent of biomass accumulation (Dring 1982). Photosynthesis consists of two major reactions. The first is the capture of light energy and its conversion to chemical

potential as ATP and NADPH during light reaction. The second group, the "dark reactions" is the sequence of reactions by which the chemical potential is used to fix inorganic carbon.

Light reaction begins when a chromophore absorbs a photon and is elevated from ground to an electronically excited state. The excited electron is more energetic and less stable and has a tendency to fall back to ground state. The simplest degradation is a reversal of high absorption by the emission of a photon or fluorescence.

The basic model of photosynthetic electron transport is the Z-scheme (Hill and Bendall 1960) which incorporates two photosystems. The electron donor for PSII is water and the ultimate electron acceptor for PSI is $NADP^+$.

The concept of a functional unit of photosynthesis was first put forth by Emerson and Arnold (1932) and a large body of research has led to a number of detailed models of the PSU (Govindjee and Braun 1974). Other important determinants of plant photosynthetic rates include the natural levels of PAR and the plant ability to capture light efficiently. Production of carboxylating enzyme is an energy "cost" measured by the level of respiration occuring in the tissue, and may account for lower respiration in "shade" plants (Bjorkman *et al* 1972). Spence (1982) points out that there are essentially no true aquatic `sun' species becuase photosynthetic rate of all aquatic plants become light saturated well below full sun light. Adaptation in pigment content and composition for the capture of available light by macrophytes and phytoplanktons are discussed by Barko and Filbin (1983), Kirk (1983) and Andrews *et al.* (1984).

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**Spectral distribution**

In the sea, incident light is exponentially attenuated in accordance with the relationship

$$I_2 = I_0 \, e^{-k_2}$$

Where $I_2$ is the PFD transmitted to depth 2. $I_0$ is the incident PFD and K is the extinction co-efficient of the specific water column. It is known from many studies (Levring 1947, Haxo and Blinks 1950), that seaweed photosynthesis varies as a function of wavelength. The action spectra from red seaweed, *Schizymenia pacifica*, shows a marked deviation from its absorptance spectrum i.e., the soret and main band chlorophyll absorption appear relatively 'inactive' compared to the regions absorbed by the biliproteins; phycoerythrin (480-580 nm) and phycocyanin (580-640 nm). This is explained by the preferential transfer of energy captured by biliproteins to PSII (Haxo and Blinks 1950). Action spectra for $O_2$ production display the degree of enhancement of a fixed wavelength background illumination by a variable wavelength beam. The resulting enhancement spectra for the red seaweeds *cryptopleura crispa* and *Porphyra perforata* in background light of 546 nm (strongly absorbed by phycobilins) no longer show the 'inactive' chlorophyll (Fork 1963). These enhancement spectra for red seaweeds show that the basis for the enhancement effect is not the pairing of low energy quanta absorbed by chlorophyll *a* with higher energy quanta absorbed by accessory pigments, but rather the requirement for dual excitation of accessory pigments and chlorophyll *a* for efficient photosynthesis.

Numerous investigations on measurable rate of photosynthesis (apparent photosynthesis and net photosynthesis) were carried out (Mathieson and Burns 1971, Buesa 1977, Kremer and Kuppers 1977, Dawes *et al.* 1978, Kremer 1979a). In general, photosynthetic carbon assimilation in seaweeds compose

well with average net photosynthetic fixation of 90-800 µmol $CO_2$ $dm^{-2}h^{-}$ or 220 - 1300 µmol $CO_2$ $g^{-1}$ dry weight $h^{-1}$ at saturating conditions (Burris and Black 1976).

Studies on several marine algae indicate that maximum photosynthetic rate of a single species is not exclusively light dependent, but are due to seasonal adaptation (Zavodnik 1973a, b, King and Schramm 1976, Brinkhuis 1977a,b, Titlyanov and Zvalinsky 1978). Seasonal fluctuations of photosynthesis in *Laminaria sp.* (Luning 1971, Hatcher *et al.* 1977) can partially be attributed to seasonally differing amounts of carboxylating enzymes (Kuppers 1978, Kuppers and Weidner 1980). Observations have shown that even diurnal rhythms of photosynthesis and photosynthetic enzymes occur in marine algae (Zavodnik 1978, Kageyama *et al.* 1979, Yamada *et al.* 1979a).

**Carbon budget**

Measurement of the primary productivity of marine macroalgae is largely a function of a number of interactions between the physiology of the organisms, the methodology used and the physics of the environment. Primary productivity of some marine algae were compiled (Chapman 1976, Topinka and Robbins 1976, Chapman and Craigie 1977, Jackson 1977, Chapman *et al.* 1978). *Macrocystis pyrifera* in the kelp beds off California was found to achieve net primary productivity exceeding about 5 g $cm^{-2}dm^{-1}$ as based on measurements of standing crop and photosynthetic carbon gain (Blinks 1955, Towle and Pearse 1973, Jackson 1977, Wheeler 1978). *Macrocystis* which attains growth rates in the range of up to 1600 g fresh weight $dm^{-1}$, equivalent to 5.7% $dm^{-1}$ (Wheeler 1978), appears to be one of the most productive plants of marine environment.

Annual carbon budgets, derived from long term *in situ* measurements throughout the year, have been obtained for *Laminaria longicruris* (Hatcher *et al.* 1977) and for *Laminaria saccharina* (Johnston *et al.* 1977). It is now well established that particularly in the temperate latitudes marine algae undergo

distinct seasonal fluctuations in growth, reproduction and a number of biochemical changes (Boney 1965, Zavodnik 1971, 1973a, b, Fuller and Mathieson 1972, Dawes *et al.* 1974).

**Photorespiration**

Photorespiration in marine algae has not been well characterized. There are data which indicate enzymatic differences as compared with higher plants e.g., glycolate dehydrogenase rather than glycolate oxidase is found in the majority of green algae tested but not in red and brown species (Frederick *et al.* 1973, Tolbert 1976). The inhibition of photosynthesis by oxygen has been found in a few of the marine macroalgae (Brown and Tregunna 1967, Black *et al.* 1976, Dromgoole 1978, Reiskind *et al.* 1988). Large gradients and diurnal changes in oxygen tension can occur in marine ecosystem (Odum *et al* 1963, Richard 1965) and these may have a significant effect on benthic algal production.

The extent to which photorespiration occurs in algae and particularly in seaweeds is a matter of controversy (Harris 1980). Rubisco is the ubiquitous enzyme catalyzing the fixation of $CO_2$ as a first step in phtosynthetic carbon assimilation. Photorespiration arises because RuBP carboxylase can also act as oxygenase, binding $O_2$ rather than $CO_2$ to RuBP. Few measurements of photorespiration indicators have been made on seaweeds (Tolbert and Osmond 1976, Burris 1977) but evidence so far suggests that it does occur, particularly under high $O_2$ concentration. The best evidence for the presence of photorespiration was demonstrated by Akazawa and Osmonds (1976). The activity of oxygenase is only 1% of that of carboxylase. However, marine plants are usually exposed to high $CO_2/HCO_3^-$ and low $O_2$, photorespiration is probably of little importance in productivity (Kremer 1981).

Kinetics of Rubisco in marine macroalgae have been reported previously

only for a few species. Recalculated Km values for $CO_2$ averaged 33 µM for four species (Kerby and Raven 1985). Cook and Colmon (1987) found a Km of 30 µM for the red algae *Palmaria palmata* while Beer *et al.* (1990) reported a value of 70 µM for the green alga *Ulva fasciata*. Comparing their relatively high Rubisco Km ($CO_2$) values with external $CO_2$ concentration (about 10 µM) it seems that marine macroalgae should have developed $CO_2$ concentrating mechanisms. The presence of such systems is strongly indicated by the $O_2$-insensitive net photosynthesis rates and low $CO_2$ compensation points found for many species (Reiskind *et al.* 1989). However $C_4$ photosynthesis has been found in some forms (Kremer 1981, Kerby and Raven 1985, Reiskind *et al.* 1988) including *Gracilaria conferata* (Israel *et al.* 1991).

The ecophysiology of macroalgae is often studied with respect to a single environmental variable. However, factor interactions such as light-nitrogen (Duke *et al.* 1986) and light-temperature (Lapointe *et al.* 1984a,b) result in more complex physiological responses than is apparent from single factor experiment. Automated photosynthesis light response (P-I) curves, based on brief incubation at series of PFDs, have allowed detailed study of diurnal photosynthesis and photoinhibitory phenomena (Henley *et al.* 1991a, b, 1992) as well as inorganic carbon requirements of photosynthesis (Levavasseur *et al.* 1991). However, interpretation of changes in P-I curves alone in the context of photoinhibition can be ambiguous. For this reason it is helpful to supplement photosynthesis measurements with analysis of chlorophyll fluorescence. Specifically, PSII has been identified as the site of photoinhibition (reviewed by Krause 1988), and PSII fluorescence properties are thus targeted. This can be performed by following the fluorescence rise from a minimum at the instant a weak continuous actinic light is applied (Fo) to a stable maximum (Fm) after all PSII centers have been oxidized. The difference of Fm-Fo is variable fluorescence (Fv). The ratio of Fv/Fm is related to light-limited photosynthetic

quantum yield ( $\phi_m$ ) (Bjorkman 1987), and changes in Fo are helpful partitioning the reduction in Fm into damage and protection components (Franklin *et al.* 1992).

**Agar**

Agar is a major constitutent of the cell wall of certain red algae, especially members of *Gelidiaceae* and *Gracilariaceae*. Until 1900, agar was used mainly as a food item. It is the first seaweed polymer extract to achieve commercial status for purposes other than food. Extraction of agar from seaweeds was discovered in Japan in 17th century. In 1881 it was used for the first time in microbiological research (Hitchens and Leikind 1939, Chapman 1970).

Agar yield and quality vary among species, populations and different stage of life cycle (Yaphe Duckworth 1971, Penniman 1977, Whyte and Englar 1978, 1979, 1981, Kim and Henriquez 1979, Usov *et al.* 1979, Asare 1980, Bird *et al.* 1981, Whyte *et al.* 1981, Yang *et al.* 1981, Craige *et al.* 1984). It is found that gel yield and quality are inversely related to seaweed densities (Asare 1980, Wang and Yang 1980, Whyte *et al.* 1981, Friedlander and Zelikovitch 1984). Experimental incubation of *Gracilaria* shows that nitrogen content of the thallus, temperature and tissue age are the important facators (De Boer 1979, Bird *et al.* 1981) for agar yield. Yield of native agars was 9-11% in young parts of *Gracilaria tikvaliae* and 19-23% from mature parts (Craigie and Wen 1984).

Agar consists of two components, agarose and agaropectin. The former is relatively simple and constant in composition, consisting of alternating units of D-galactose and 3,6-anhydro L-galactose, none of which are sulphated. Agaropectin has the same basic structure, but with a variable degree of substitution by sulphate, pyruvate and other groups (Booth 1975). It is the ratio between agarose and agaropectin that varies from species to species, and the gel strength of the agar is proportional to its agarose content.

Industrial applications of agar are dominated by three quality grades:

a) Sugar reactive agar whose gels are consistently stronger as a function of sugar concentration

b) Standard agar having the temperature and other requisites for microbiological purposes

c) Food grade agar which is any agar not meeting the standard for (a) or (b). In *Gracilaria* the sulfate level of the agars depends on both environmental conditions and on the species (Doty and Santos 1983).

In the previous studies, seaweed growth rates in the field have been shown to be highly variable due to fluctuation of natural factors (Rama Rao 1970, Dawes *et al.* 1974, Durako and Dawes 1980, Guist *et al.* 1982). Furthermore, phycolloid extracts such as agar and carrageenan are known to be affected by seasonal changes (Dawes *et al.* 1974, Mshigeni 1976, Hoyle 1978, Simpson and Shachlock 1979).

At present there are about 30 agar and 28 algin industries situated in different parts of India. They depend on the raw materials collected from natural seaweed beds of Tamil Nadu coast (Kaliaperumal *et al.* 1989).

**Other biochemical constitutents**

Chemical analysis have been carried out on marine algae especially those having economic importance. In red alga *Eucheuma* in Florida, Dawes *et al.* (1974) demonstrated that the ratio of protein to carbohydrate could be used to determine whether the plant is in rapid growth phase (1:10), slow growth phase with high photosynthetic activity (1:16), or in reproductive phase with no growth (1:25). It is observed that deep water plants had higher level of protein (6-8% of dry weight) than shallow water plants (2-5% of dry weight). Lipid is present in a limited quantity in marine algae but marine algal lipids contain a wide array of major fatty acids and show variation from higher plants.

The red algae constitute high levels of $C_{20}$ poly unsaturated fatty acids (PUFA) mainly eicosapentaenoic acid (EPA) and arachidonic acid (Khotimchenko and Svetashev 1987). Other abundant fatty acids in this class are palmitic acid, palmitoleic acid and oleic acid.

**Seaweed and their uses**

The abundance and diversity of seaweeds have made them prime material for human use. It is used as food, fodder, manure, drugs and for industrial purposes. The emergence of the modern seaweed chemical industry started with the extraction of basic chemicals as soda, potash and iodine. In Russia, the red seaweed *Phyllophora nervosa* very abundant in the Black sea, was harvested for electrolytic separation of iodine, bromine and chlorine in 1930. The industry based on the extraction of small molecules from seaweeds experienced declined when the chemical extracted from seaweeds could be obtained at less expense from alternate sources, like agar and agar, carageenan and algin.

**Mariculture of Gracilaria**

An annual production of 25,000 - 30,000 metric tonnes of *Gracilaria* is harvested from the wild in Chile, Argentina, Brazil and South Africa and from fish pond culture in Taiwan and China (Santelices and Doty 1989). In pacific Asia, the Philippines is one of the producers of *Gracilaria* mainly from natural stock.

Steady increase in market demand together with lack of crop management have led to over harvesting the natural stocks, shortage of *Gracilaria*, higher prices for the crop and demand for reliable crop quantity and quality. The outcome has been of great interest in *Gracilaria* farming and a diversity of farming methods has been developed.

Commercial pond farming of *Gracilaria* is successful in Hainan, China (Wang *et al.* 1984, Liu 1988) and Taiwan (Shang 1976, Chiang 1981). Encouraging results were obtained from cultivation of *Gracilaria* on lines (Kim and Haumm 1965; Raju and Thomas 1971, Smith *et al.* 1984, Doty 1986, Camara-Netto 1987, Barraca 1990, Hurado-Ponce 1990), rafts (Li *et al.* 1984, Ren *et al.* 1984) floating bags (Saunders and Lindsay 1979), raceway (Fralick *et al.* 1981) and tanks (Lapointe *et al.* 1976, Lapointe and Ryther 1978, Lignell *et al.* 1987).

Besides the vegetative propagation of *Gracilaria* in ropes, net, tanks and ponds, reproductive propagation was also adopted (Killian 1914, Oza and Krishnamurthy 1967, Rao and Thomas 1974, Oza 1975, Bird *et al.* 1977a, Jayasankar *et al.* 1991, Jayasankar 1990, 1992). Spore attachment on new lines placed in dense wild crops was successful (Smith *et al.* 1984) and seemed practical in West Indies. In Malaysia also successful spore culture of *Gracilaria* was established.

It was also suggested (Cheney 1984) that to make seaweed cultivation commercially more attractive, it was necessary to establish improved strains superior in quality and quantity or greater dependability. The classical example for genetic improvement of seaweed is *Laminaria japonica* which was originally a cold temperate seaweed and now extensively cultivated in subtropical waters of China (Fang *et al.* 1978, Tseng 1981). Subsequently, intraspecific crosses were reported in *Gigartina* (Polanshek and West 1977), *Porphyra* (Miura 1979) and *Eucheuma* (Doty 1979). Due to sexual incompatibility and infertility between the different species (Chen and Taylor 1980, Guiry and West 1983), somatic hybridization and cell transformation techniques using protoplast have been developed (Cheney 1984).

Protoplast research in seaweed is a relatively new field and lags far behind the land plants and unicellular algae (Berliner 1981, 1983, Cheney 1986). Viable protoplasts have been produced in two genera, *Porphyra* and *Gracilaria* of red algae ( Tang 1982,Saga and Sakai 1984, Polne-Fuller and Gibor 1984, Fujita and Migita 1985, Saga *et al.* 1986). So far, protoplast culture of *Gracilaria* to regenerate whole plants have been unsuccessful (Cheney and Mar 1986).

# Materials and methods

**Geographical conditions**

Tamil Nadu is situated on the South-east coast of India and Mandapam lies between 78° 08'E and 9° 17'N in between Palk Bay and Gulf of Mannar (Fig. 1). Both the seas are parts of Bay of Bengal but show different environmental conditions influenced by monsoon periods. Sea conditions in the Gulf of Mannar are similar to those of South-west coast. Sea is turbulent during June to September due to South-west monsoon. The Palk Bay is generally calm during this season. North-east monsoon (October - February) makes sea condition in the Palk Bay turbulent while the Gulf of Mannar is fairly calm. Gulf of Mannar is characterised by the presence of a chain of 21 islands, relatively of greater depth and higher productivity compared to the Palk Bay. .

**Description of study area**

Considering the location specificity of seaweeds in their distribution, two centers, namely Pudumadam and Thonithurai were selected for collection of samples in the Gulf of Mannar.

Pudumadam is about 20 Km from Mandapam (9°17'N and 79°'E) having a rocky coast and sandy bottom (Plate 1). *Gracilaria corticata* grow abundantly here either attached to the rocks or to the sandy bottom (Plate 2). The sea is rough due to open sea impact.

Thonithurai is situated about 8 Km from Mandapam (9°17'N and 70° 11'E). The coast is sparcely rocky and the sea bottom is muddy covered by seagrasses. *Gracilaria edulis* and *Gracilaria crassa* grow well in this area. Sea off Thonithurai is relatively calm due to presence of islands which blocks strong wave action (Plant 3).

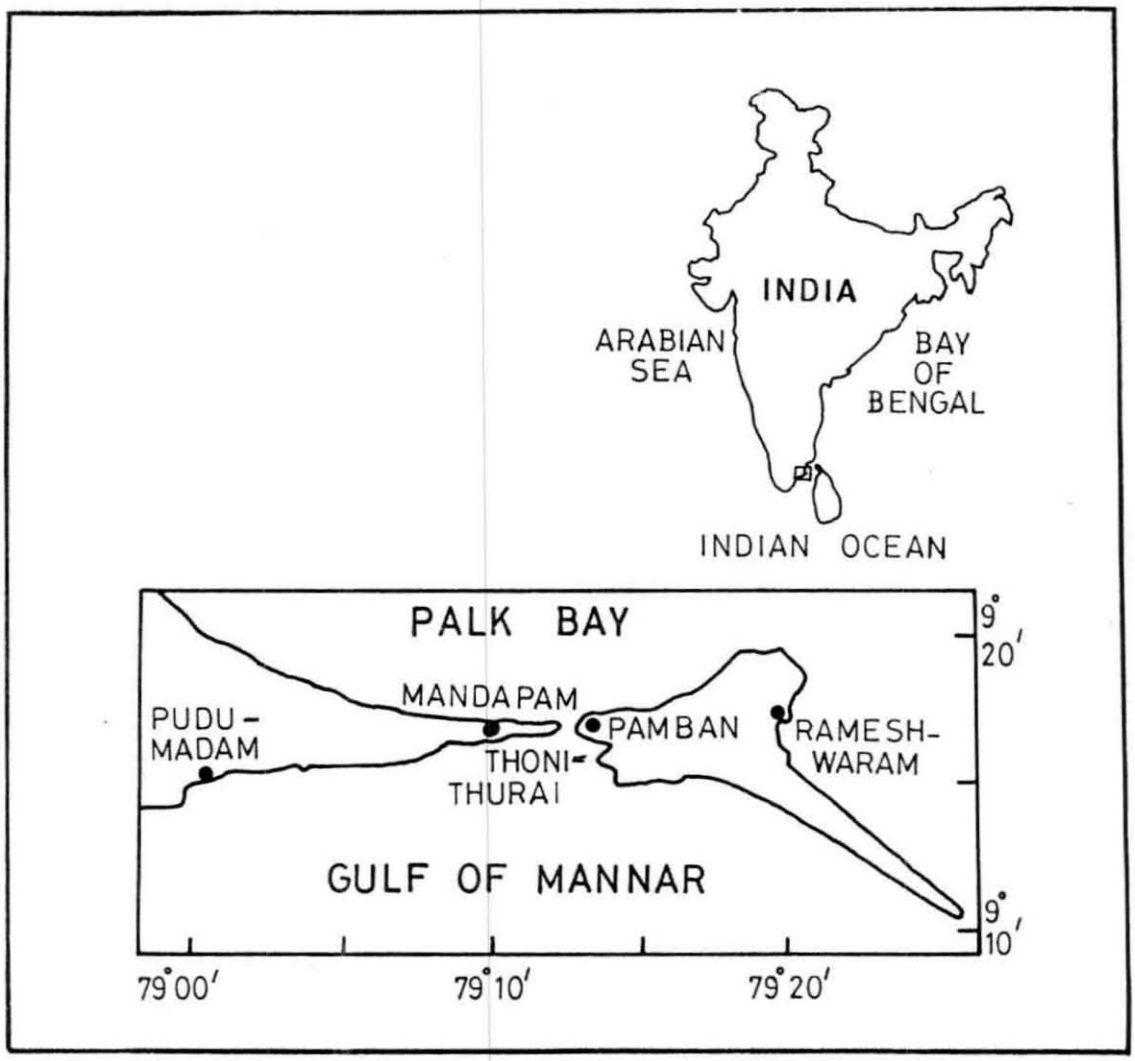


**Fig. 1**

The major collection sites, Thonithurai and Pudumadam situated in the South east coast of Tamil Nadu, India.

**Plate 1**

Pudumadam, the collection site for *G. corticata* in Gulf of Mannar, South east coast of India.

**Plate 2**

Rocky shore of Pudumadam showing luxuriant growth of *G. corticata* attached to rocks.

**Plate 3**

Thonithurai, collection site for *G. edulis* and *G. crassa* in the Gulf of Mannar, South east coast of India.

**Metereological data**

Metereological data such as rainfalls, sunshine, humidity percentage, maximum and minimum temperature were collected from the metereological center, Pamban.

**Sampling of seaweeds**

*Gracilaria edulis*, *G. crassa* and *G. corticata* were collected from specific areas of availability during low tides in the morning. Quadrat sampling of seaweeds was done by placing three quadrats (0.5 $m^2$) randomly in different areas. Then they were cleaned of epiphytes, epifauna, pebbles and other dead gastropod shells, washed in tap water, blotted and fresh weight was taken. Biomass was expressed in gram fresh weight per square metre.

Few plants from each species were collected separately, washed thoroughly and transported to laboratory (Department of Plant Sciences, Madurai Kamaraj University, Madurai, situated 160 Km from the area of collection) in enriched seawater to study different physiological parameters.

**Dry weight**

The quadrat sampled plants were dried in an oven at 105°C for 2-3 days. Dry weight was taken nearest to 1 mg and percentage of dry weight was calculated.

**Pigment estimation**

Estimation of pigments such as chlorophyll *a*, phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) was carried out by the procedures of Jefferry and Humphery (1975).

**Chlorophyll a**

Known quantity of plant material was cut into small pieces, ground in a pre-cooled mortor and pestle with 90% acetone. The extract was centrifuged

at 5,000 g. Absorbance of the clear supernatant was taken at 663 and 630 nm in a Hitachi 557 spectrophotometer.

**Phycoerythrin, phycocyanin and allophycocyanin**

Extraction of the phycobiliprotein was carried out in 0.1 M phosphate buffer at pH 6.5. Known quantity of fresh algal material was cut into pieces, ground in a pre-cooled mortor and pestle in the above buffer. The extract was centrifuged at 7,500 g in a refrigerated centrifuge at 4°C for 10 min. The absorbance of the supernatant was taken at 498, 615 and 652 nm in a Hitachi 557 spectrophotometer. Estimation of pigments was carried out using the following formulae :

Chl a = (11.47 x A663) - (0.4 x A630)

PE = (155.8 x A498) - (40.4 x A615) - (10.5 x A652)

PC = (151.1 x A615) - (99.1 x A652)

APC = (181.3 x A652) - (22.3 x A615)

**Field photosynthesis measurements by Winkler's method**

Photosynthetic activity of seaweeds was measured by Winkler's method in the field immediately after collection (Strickland and Parsons 1968).

Two pairs of light and dark bottles (125 ml capacity) were filled to filtered seawater. Few plants were put in one light and one dark bottle, whereas the other set of light and dark bottles were kept as such with filtered seawater to note the background photosynthesis and respiration. One light bottle was taken to find out the dissolved oxygen content of seawater before the experiment. After one hour of exposure under outdoor environment, the dissolved oxygen was fixed by manganous sulphates and alkaline iodide solution. The precipitate formed was allowed to settle to half the volume and was dissolved by adding

concentrated sulphuric acid. Fifty ml of aliquots were taken from each bottle and titrated against standard thiosulphate solution. The rate of net photosynthesis and net respiration was estimated by following formulae:

1. Net photosynthesis = (light bottle + algae) -(Blank bottle)
2. Net respiration = (Blank bottle)-(Dark bottle + algae)
3. Background photo-synthesis = (Light bottle, blank) - (Dark bottle, blank)
4. Background respiration = (Blank bottle) - (dark bottle, blank)

Net photosynthesis and net respiration rates were expressed as ml of $O_2$/g DW/h.

**Determination of photosynthetic rate by oxygen electrode**

After transporting the sample to laboratory, they were maintained in the growth chamber at 25°C and 16L:8D photoperiod to overcome the transportation stress.

The apical portions of the plants were hanged from the top inside the cylindrical oxygen electrode chamber (Hansatech, UK) containing 2 ml of filtered seawater. Saturated white light of 100 $Wm^{-2}$ was passed through a round bottom flask (10 cm dia water bath) from the slide projector (Photophone Ltd., India) before illuminating the chamber. The water inside the cylindrical tube was stirred continously by a magnetic stirrer. The amount of oxygen evolved was monitered continuously at 25°C. Mean of three consequitive reading were taken for calculation. Rate of photosynthesis was expressed as µmol $O_2$ evolved/ g fresh weight/h.

**Estimation of trace elements**

Determination of trace elements such as K, Ca, Mg, Mn, Na, Cu, Pb, Fe and Zn were carried out by atomic absorption spectrophotometry with a flame inonization detector.

Two hundred and fifty mg of dried algal powder was taken in a 75 ml digestion tube. The samples were digested in triple acid mixture (5 ml of $HNO_3$ + 2 ml of $HClO_4$ and 0.5 ml of $H_2SO_4$). The tubes were placed on a digestion block until acid mixture become colorless. They were cooled to room temperature, diluted to 50 ml with deionized water and filtered through Whatman No. 42 filter paper.

Elemental analysis was performed by flame atomic absorption spectrophotometry using recommeded guide lines for wavelength selections and linear working range (Perkin Elmer 1982). For Ca, K, and Na further dilution of the extract was made with deionized water.

**Estimation of total protein**

Total protein was estimated by the method of Lowry *et al.* (1951).

Ten mg of dried algal powder was taken in a test tube containing 5 ml of 1N NaOH, capped with marble and allowed to stand for 24 h at room temperature. The tubes were then vortexed, centrifuged at 5,000 g for 5 min and 0.5 ml of the extract was pipetted out to separate tubes. Three mililiters of freshly prepared alkaline copper tartarate reagent was added to each tube followed by 0.5 ml of Folin's reagent diluted with distilled water (1:2), vortexed and allowed to stand for 20 min to develop colour. Absorbance was measured at 750 nm in a Hitachi 557 spectrophotometer. BSA was used as the standard.

**Estimation of total carbohydrate**

Total carbohydrate was estimated by phenol sulfuric method (Dubois *et al.* 1956).

Five mg of dried algal powder was taken in a test tube containing 10 ml of 5% TCA. The tubes were heated in a water bath at 80-90°C for 3 h. After cooling cooled to room temperature, the volume was made up to 10 ml with distilled water. From the extract 0.2 ml was pipetted out to a 10 ml

test tube. One mililiter of 5% phenol was added to it, after mixing gently 5 ml of concentrated sulphuric acid was added. Then the samples were cooled to room temperature and absorbance was measured at 490 nm in a Hitachi 557 spectrophotmeter. Sucrose was taken as the standard. The total carbohydrate content was expressed as percentage.

**Estimation of total lipid**

Total lipid content was estimated by phosphovanillin method (Barnes *et al.* 1973) after extracting the lipid by Folch *et al.* (1957) method.

**Extraction of lipid**

Fifty mg of dried algal powder was taken in 12 ml screw capped test tube containing 10 ml of chloroform:methanol (2:1). The tube was loosely capped and heated for 30 min at 60°C in a water bath. After complete extraction, the volume was made up to 10 ml with chloroform:methanol (2:1) and filtered. From the extract 0.4 ml was taken in a 7 ml test tube, allowed to dry completely and digested with 0.4 ml of concentrated sulphuric acid by boiling in the water bath for 10 min.

**Estimation of lipid**

From the digested sample 0.2 ml was taken in a different test tube. Five ml of phosphovanillin reagent was added to it, kept for 30 min until the colour developed. Absorbance was measured at 520 nm. Cholestrol was taken as the standard and total lipid was expressed in percentage.

**Extraction of Agar**

Agar was extracted from the dried algal sample. Five g of dried and ground algae was treated with 40 ml of 3 N NaOH and heated at 90°C in a water bath for 1 hour. The treated algae were washed three times with 250 ml of distilled water over a plankton net. Algae were then immersed in 100 ml of 0.1 M $PO_4$ buffer (pH 6.0) over night. Next day the pH was brought

to 6.5-7.0 and then autoclaved at 110°C for 1 hour. The suspension was filtered and the filtrate was allowed to gel at room temperature, cut into pieces and kept in a freezer over night. The frozen filterate was thawed and filtered through a plankton net. The precipitate was suspended in 100 ml of isopropyl alcohol for 15 min and filtered through a plankton net. The precipitate was allowed to dry overnight. Dried agar was weighed and expressed as percentage of agar yield on dry weight basis.

**Gelling temperature**

Dried agar equivalent to 1.5 g was taken in a beaker dissolved in 100 ml of distilled water and boiled till an uniform suspension was formed (1.5% agar). The agar was allowed to set at room temperature. Gelling temperature was determined with the help of a thermometer following the movement of glass beads in gelling agar.

**Melting temperature**

The gelled agar was allowed to melt slowly on a hot plate. Circular glass beads of 1 mm size were put into the beaker, and the melting point was noted with the help of the thermometer following the movement of glass beads in the melting gel.

**Gel strength**

The agar solution (1.5%) was allowed to set overnight at room temperature. Gel strength was determined using a gelometer described by Funaki and Kojima (1951) by adding different weight to the pan of the gelometer till the gel breaks and the iron rod of the gelometer pierced completely the gel.

**Determination of dissolved oxygen content of seawater**

During each sampling period, seawater was filled up in a 125 ml BOD bottle, capped tightly after adding manganous sulphate and alkaline iodide solution (as described earlier). The precipitated formed were allowed to settle

half the volume of the bottle, and then dissolved by adding concentrated sulphuric acid. Aliquots of 50 ml were taken and titrated against standard thiosulphate solution. The dissolved oxygen content of seawater was expressed as ml $O_2$/l.

**Determination of salinity of seawater**

Salinity of seawater was determined at each sampling period by the method Strickland and Parson (1968). Ten ml of standard seawater was taken in a spoutless conical flask. Four drops of potassium chromate were added to it and titrated against standard silver nitrate solution. Salinity of sample seawater was also determined by the following formulae.

$$\text{Salinity of sample seawater} = \frac{V2}{V1} \times S$$

Where $V_1$ is the volume of standard seawater at end point and $V_2$ that of sample seawater S is the salinity of standard seawater (19.38%).

**Qualitative analysis of lipid**

Qualitative analysis of lipids was carried out by gas chromatography by the method Miller (1984).

Fresh algal sample equivalent to 100 mg was ground in 2 ml of 1.2N NaOH in 50% aqueous methanol. Sample was boiled for 30 min in a sealed tube, cooled and acidified with 0.6 ml of 10 N HCl solution with the addition of 1.0 ml of 12% $BF_3$ in methanol reagent. The sample was heated for 10 min at 85°C. Extraction of fatty acid methyl ester was done by adding 1ml of hexane and diethyl ether (1:1). Organic phase of the extract was removed and shaken with 3.0 ml of 0.3N NaOH. Again the organic FAME extract was transferred to an 1.0 ml Eppendorf and evaporated to dryness. To this 500 µl of hexane was added and vortexed, from which 2 µl was injected to GC.

**The chromatographic conditions are maintained as follows:**

| | |
|---|---|
| Oven temperature | 100° |
| Initital temperature | 100° |
| Initial time | 10°C/min |
| Rate | 10 |
| Final temperature | 160° |
| Final time | 30 min |
| Oven maximum temperature | 200°C |
| Injection temperature | 180°C |
| Detector temperature | 180°C |
| Equilibrium | 3 min |

The methyl ester fatty acids were analysed using gas chromatograph (Hewlett Packard, Model 5890A, USA) with flame ionization detector. The column (6 ft) was packed with 10% diethylene glycol succinate on chromosorb A. The flow rate of $N_2$ and $H_2$ gases were 30 ml/min.

**$^{14}CO_2$ uptake**

Experiments were conducted on photosynthetic uptake $^{14}CO_2$ among the three species of *Gracilaria* by the modified method Peschek (1978).

Fresh algal tissues equivalent to 100 mg were cut into small pieces and put in small glass vials containing 5 ml of filtered seawater. The samples were kept in a water bath maintained at 30°C and exposed to white light (100 $W.m^{-2}$) to facilitate steady state photosynthesis for 5 min. At the end of incubation 50 µl of $H^{14}CO_3$ (0.5 MBq) was added. The reaction was allowed to continued for 15 min. Care was taken to expose all the parts of the plant material uniformly to light. The algal samples were taken out, washed

thoroughly ground and centrifuged at 5,000 g. From the supernatent 10 ul was air dried on a Whatman No. 3 filter paper, put in a screw capped glass scientillation vial containing 5.0 ml scintillation liquid (SCINTO - O, United Technologies, Packard). Counts were taken with a help of a liquid scintillation counter (Packard Model 4000).

**Effect of light intensity on algal physiology**

Fresh samples of *G. edulis*, *G. corticata*, *G. crassa* were collected from the specific collection sites and transported to laboratory after cleaning thorougly. Transportation was done with care in enriched seawater at an optimal temperature. Samples were maintained for a day in growth chamber at 25°C and light intensity of 2 $W.m^{-2}$ and photoperiod of 16L:8D were provided to overcome transportation stress before starting the experiments.

The algae were exposed to different light intensities (High light 3 $W.m^2$, intermediate light 2 $W.m^2$ and low light 0.5 $W.m^2$) in a growth chamber at 25°C and a photoperiod 16:8. Enrichment of seawater was done by Walne's medium and seawater was changed at weekly interval. Observations on physiological parameter were taken just before the treatment 6 and 12 days after treatment. Changes in the absorption spectrum of the thallus, photo-synthetic activity, pigment content and fluorescent kinetics were studied.

**Effect of light quality on algal physiology**

Experiment was conducted in the laboratory conditions to find out the changes in physiological status of algae exposed different wavelengths of light. All the three species were exposed to different lights such as red (>600 nm), green (450-610 nm) and blue (390-570 nm) in a growth chamber at 28°C and photoperiod 16L:8D. Plants kept under white light (2 $W.m^{-2}$) were used as control. Observations were made on 6th and 12th day of treatment.

**Effect of salinity on algal physiology**

*G. edulis* and *G. Crassa* were exposed to different salinity levels such as 15, 25, 35 and 45%. Light and temperature were kept constant throughout the experiment. Observations were made on the changes in the photosynthetic pigments, fluorescence and absorption spectrum of the thallus on 6th and 12th day after treatment.

**Absorption spectrum**

Absorption spectra of the thallus were recorded at room temperature (25°C) using a Hitachi 557 spectrophotometer. The ground glass sides of matched cuvettes were kept in the light path so that reference and sample beams are scattered to the same extent. The slit width of the measuring beam was narrowed down to 2.0 nm.

**Fluorescent transients**

*In vivo* Chl *a* fluorescence transients were followed in intact thallus after a brief incubation period in dark. The plant samples were excited with broad band blue light (400-620 nm, Corning, CS4-96) at a photon flux density of 100 $W.m^{-2}$. The photomultiplier (Hamamatzu R376) placed at 90° to the excitation beam was protected by an interference filter ( $\lambda$ max 690 nm, half band width 12 mm, Schott, Germany). The signal from the photomultiplier was directly displayed either on a recorder (Hitachi model 056) or stored in a digital oscilloscope (Iwatsu SR 100, Japan). The signal was triggered with the help of an electric shutter with an opening time of 10 ms. Thalloid samples were placed diagonally in a 4 ml glass cuvette to face the photomultiplier at 45°C.

# Chapter I

# Results

The three species of *Gracilaria* studied in the present work exhibit wide variations in their morphology. *G. edulis* is cylindrical, regularly branched, either di-or polychotomously. *G.corticata* is dichotomously branched, thick, flattened and cartilagenous in nature. *G. crassa* is cylindrical with club shaped or oblong articulation in dichotomously and irregularly branched thallus. The latter species forms dense cushion on the substratum, growing mostly on pebbles and dead gastropod shells (Plate 4).

**Biomass**

Quadrat sampling of *Gracilaria* gave information on the growth and biomass, which varied among the species and seasonally within the species. Biomass was found to be more in *G.corticata* than in *G. edulis* and *G. crassa* throughout the year. It was found to be low during the month of May in all three species. *G.edulis* showed bimodal growth pattern with peaks in January and July. Mean length of the plant and biomass showed significantly positive correlation ($r = 0.78$). In *G. crassa* biomass was found to be maximum during November (1266 $g/m^{-2}$) and then showed a decline till the month of May. Further increase was observed from July. In *G.corticata*, biomass of the plant was more during the month of September and then declined. High growth was observed during March (1087 $g/m^{-2}$) and July (985 $g/m^{-2}$). Significant positive correlation exhibited between biomass and mean length of the plant ($r = 0.87$). Regularity in seasonal variation of growth was lacking in *G. corticata* with different growth phases and different sizes of plants found throughout the season. However, the percentage of vegetative and reproductive stages varied seasonally (Fig. 2).

**Dry weight percentage**

The dry weight percentage of *Gracilaria* varied from 10 to 20% of the fresh weight. In *G. edulis*, the percentage of dry weight was low during peak growth period (November) and the increased gradually till July. During the

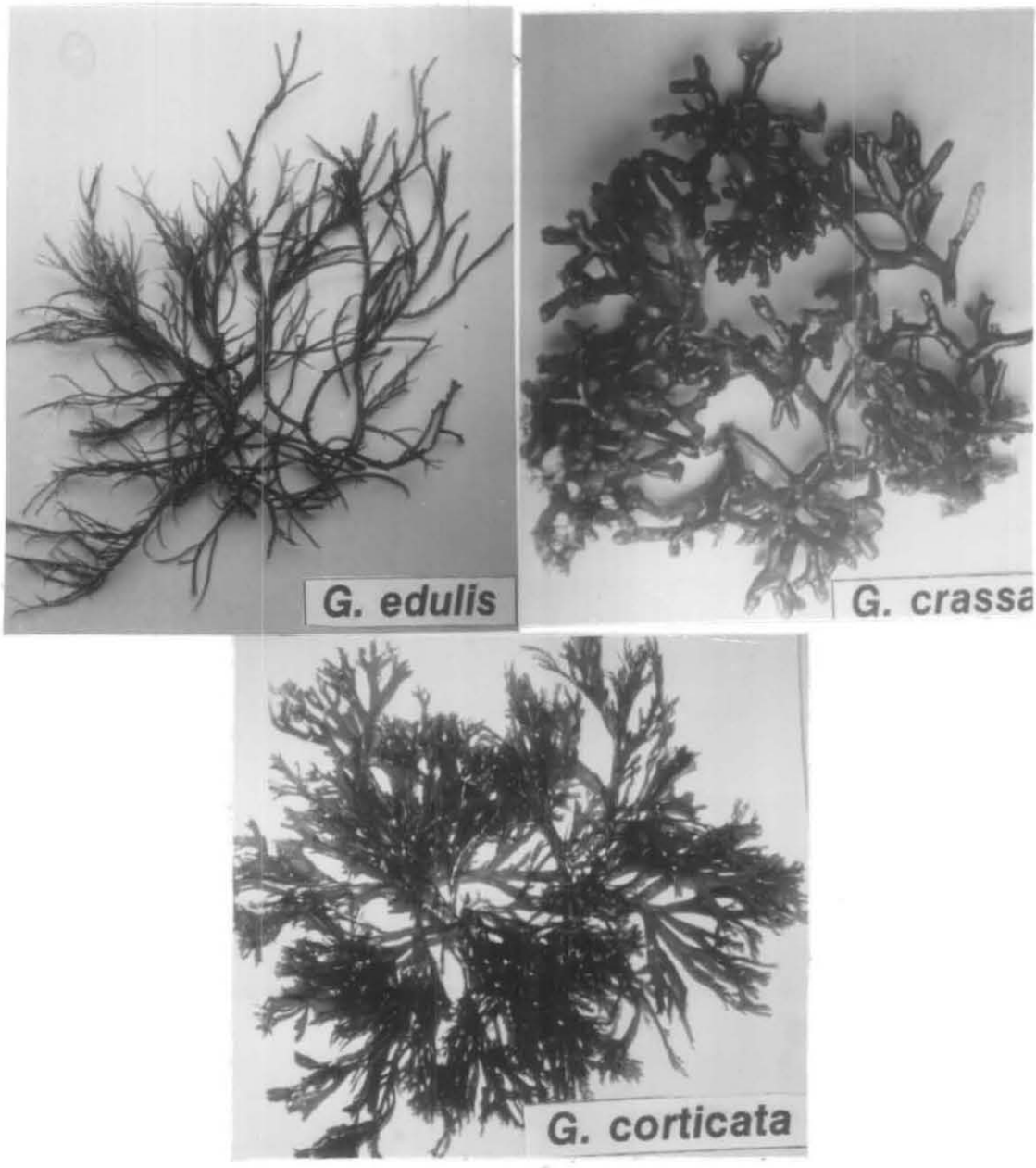


**Plate 4**

Samples of *Gracilaria edulis, G. crassa* and *G. corticata* used for the present study.

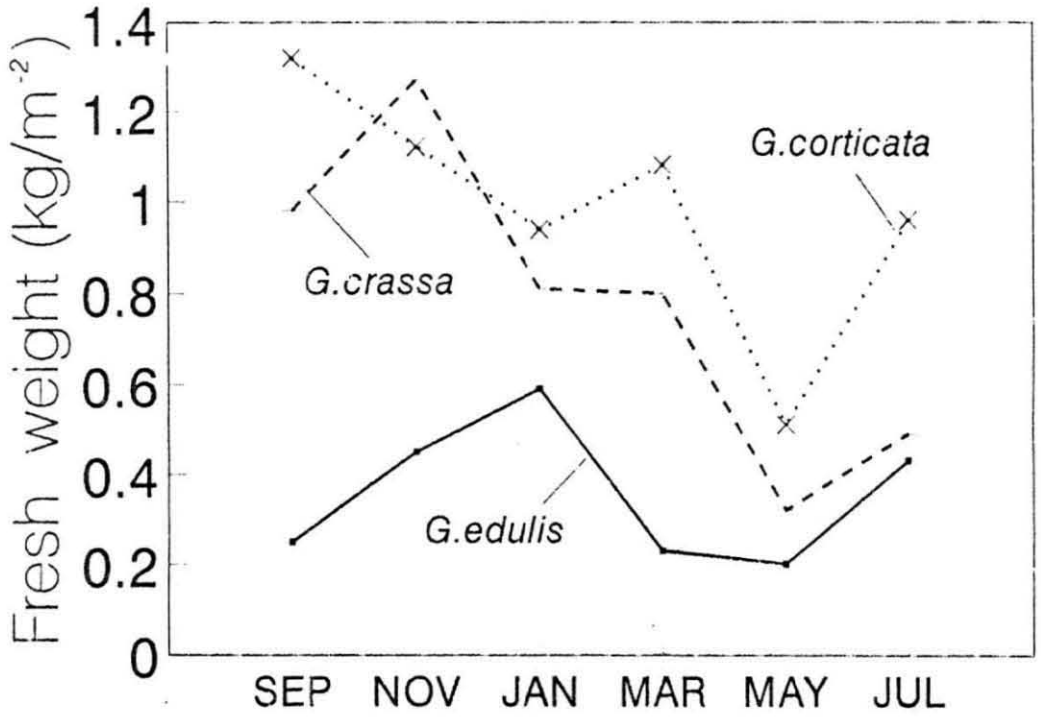


**Fig. 2**

Changes in the biomass of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. Mean values of fresh weight expressed in $Kg/m^2$. Values represent average of three quadrat samples.

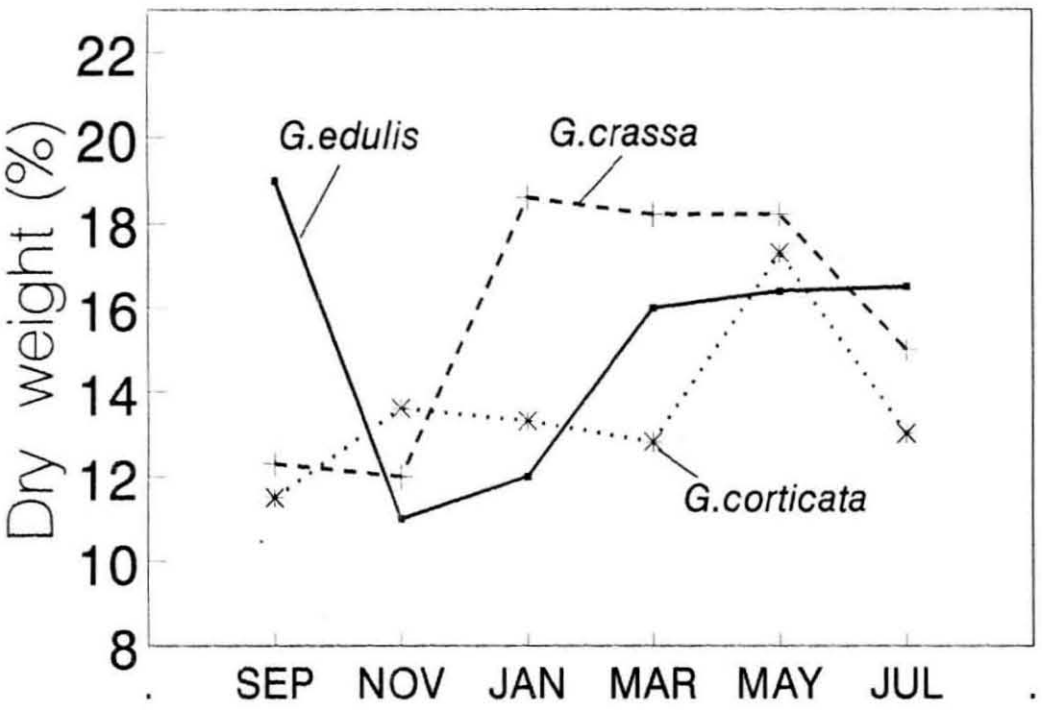


**Fig. 3**

Changes in dry weight of *Gracilaria edulis, G. crassa* and *G. corticata* collected from specific area of Gulf of Mannar at different period of the year. Mean values expressed in percentage. Values represent average of three quadrats.

lean period of growth the dry weight percentage varied from 16 to 19%. *G. crassa* also showed a similar trend. The dry weight percentage appeared to demonstrate a reciprocal relationship with biomass of the plants. Lowest value was obtained during November (12.03%) and then increased from January. In *G. corticata* there was no marked variation in the dry weight percentage of plant throughout the year, though it showed almost a similar trend in other two species with peak during May. Negative correlation existed between growth and dry weight percentage. Lowest value was obtained during September (11.49%) when the growth rate was found to be maximum (1320 $g/m^2$). Further, during May the growth rate declined to 510 $g/m^2$ when the percentage of dry weight was maximum (Fig. 3).

**Pigment content**

Red algae are predominated by the accesssory pigments such as PE, PC and APC present in PBS. Among Chl pigments, red algae contain exlusively Chl *a*, though some reports suggested the presence of Chl *d* also (Dawes 1981).

Chl *a* content of *Gracilaria* species showed similar pattern of variation like growth. Maximum Chl content was observed during January (101.3 µg/g FW)in *G. edulis*, when the growth rate was found to be highest and then declined till March with marginal increase during May. Further it increased in July (98 µg/g FW) showing a distinct bimodal pattern. In *G. crassa*, the chlorophyll content was found to be maximum (109 µg/g FW) during November corresponding to the peak period of growth and declined gradually till May. Further it increased marginally during July. In *G. corticata* Chl content did not show any regularity in its variations. However, it exhibited a similar trend like growth with a highest level in September (252 µg/g FW) and lowest in May (98.7 µg/g FW). Chl content was found to be maximum in *G. corticata*

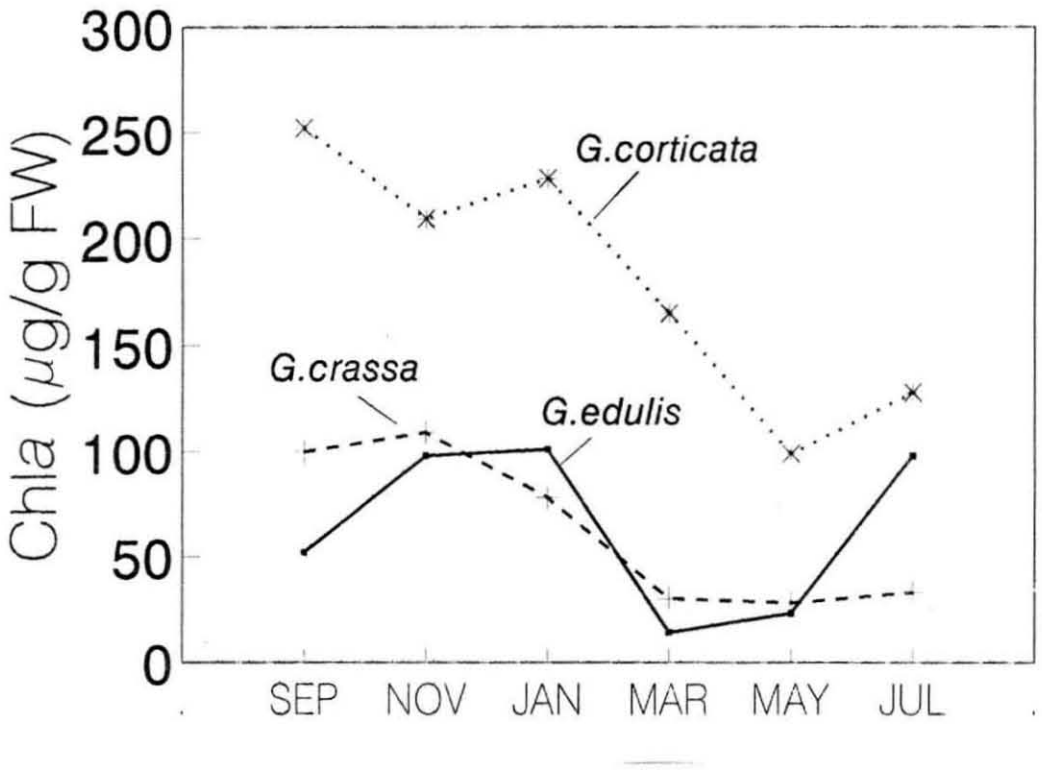


**Fig.4**

Changes of the chlorophyll content of *Gracilaria edulis, G. crassa* and *G. corticata* collected from specific area of Gulf of Mannar at different period of the year. Mean values expressed in μg/g FW. Values represent average of three samples.

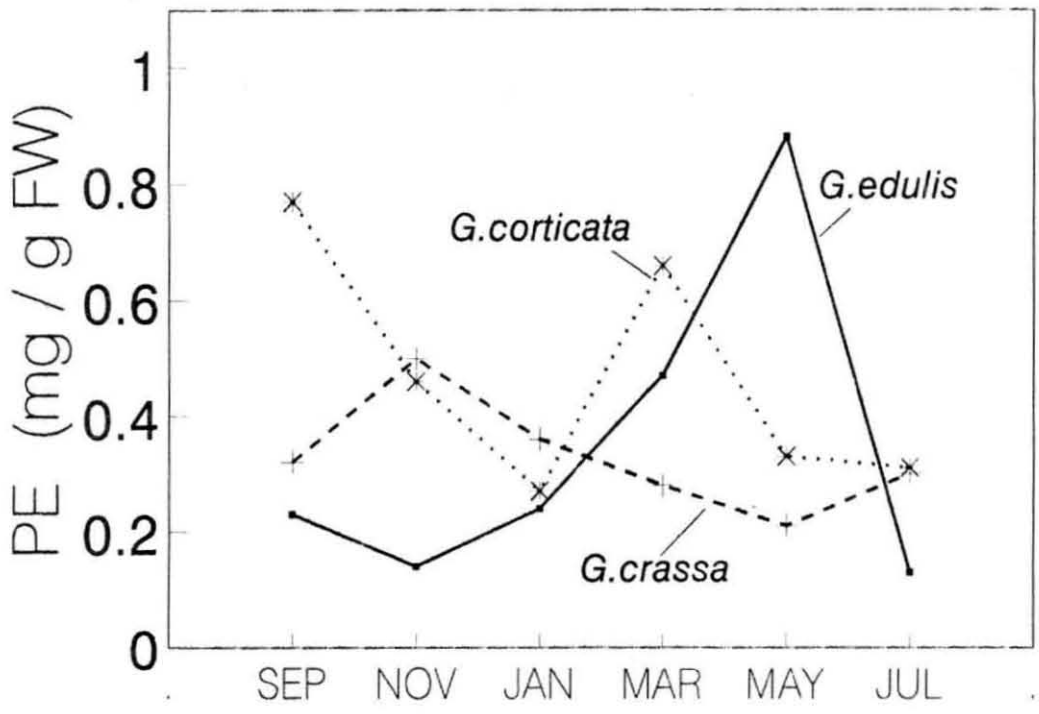


**Fig. 5**

Variation in the Phycoerythrin content of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. The mean values are expresssed in µg/g FW. Values represent average of three samples.

throughout the season than other two species of *Gracilaria* (Fig. 4). In all the species positive correlation was observed between growth and Chl content.

The accessory pigment showed different patterns among the species. PE content of *G.edulis* varied from 24 to 874 µg/g FW and showed significant negative correlation with growth and Chl content. It declined marginally from September to November and then increased till May (87.4 µg/g FW), when the growth rate was lowest. Further decline of PE content was noticed in the month of July. In *G. crassa* the PE content varied in the same manner as Chl content and growth and exhibited a significant positive correlation with growth and biomass of the plant. PE content was found to be highest in November (502 µg/g FW) and lowest in May (203.5 µg/g FW). From November to May, there was a gradual decline of PE content and thereafter it had increased. In *G. corticata* the PE level was found to be maximum (770 µg/g FW) in September corresponding to the peak growth period. Further, it declined till July although there was some marked increase of PE in the month of March showing a bimodal pattern. There was no relationship of PE with chlorophyll content and growth (Fig. 5).

Phycocyanin content of *G.edulis*, ranged from 94 to 491 µg/g FW. Maximum PC content was seen in May when the growth rate was lowest. The PC content increased from September to March and peaked in May followed by a decline in July. In *G.crassa* the PC content declined from September to January and then increased to the maximum in May (195 µg/g FW). Maximum PC content was noticed during the low growth period but no significant negative correlation existed between growth and PC content. PC content of *G.corticata* showed a trend different from those of *G. edulis* and *G. crassa*. There was no regularity of its variation throughout the season with a peak in March (Fig. 6). Maximum PC content was obtained in March (359 µg/g FW).

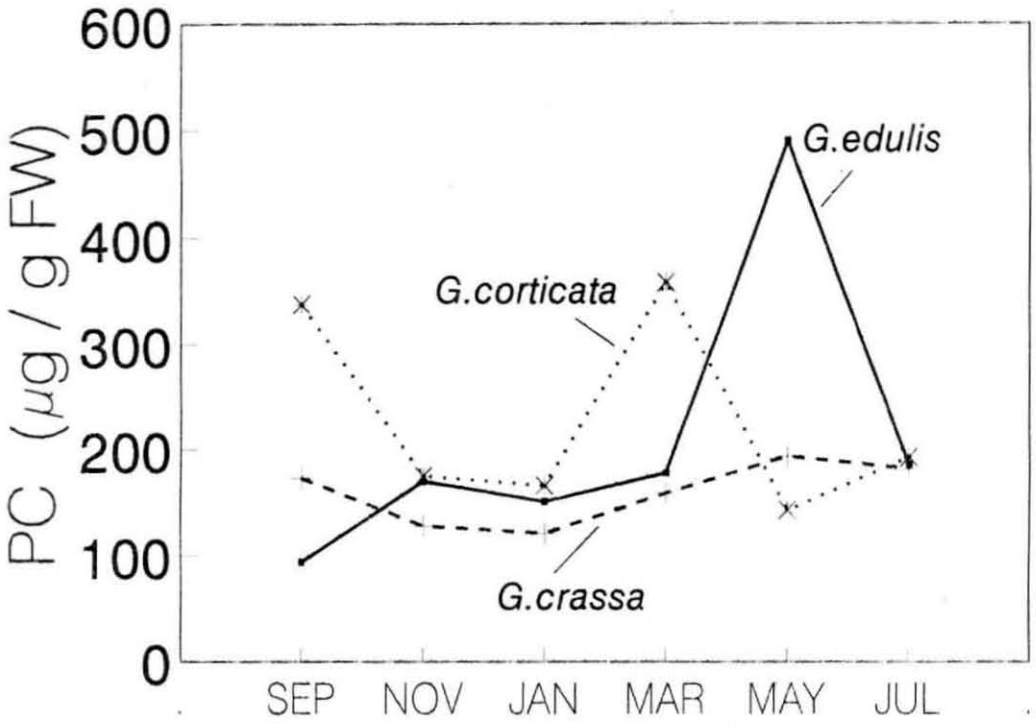


**Fig. 6**

Variation in the Phycocyanin content of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. The mean values are expressed in µg/g FW. Values represent average of three samples.

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Allophycocyanin content of *G. edulis* showed significant negative correlation with growth (r = -0.78). Lowest value of APC was observed in January (122 µg/g FW) and the maximum in May (460 µg/g FW). There was a gradual decline of APC from September to January and then increased till May. Further decline was observed in July. In *G. crassa* no significant correlation could be noticed between APC and growth. The APC content declined marginally from September to November followed by an increase in the month of January and then declined till July. It ranged from 117 to 445 µg/g FW. APC content was found to be minimum in July. *G. corticata* too showed a similar pattern like that of *G. crassa*. APC content declined from January to May and then increase further (Fig. 7). It did not exhibit significant correlation with growth and other pigments. Maximum APC content was observed in Januray (509 µg/g FW) and minimum in May (109 µg/g FW).

**Net photosynthetic activity**

The rate of photosynthesis in *Gracilaria* showed wide interspecific and seasonal variation. In *G. edulis*, the activity was found to be maximum during November (3.7 ml $O_2$/g DW/h) and gradually declined till March. Further, it increased till July. The field photosynthetic activity showed significant positive correlation with growth (r = 0.83). In *G. crassa*, the photosynthetic activity declined gradually from September to March and again increased upto May. *G. corticata* did not show much variation in the photosynthetic activity throughout the year. Maximum and minimum activities were noted during September and May, respectively and was significantly correlated with growth (r = 0.97). Among the three species studied in the present work, *G. edulis* exhibited maximum rate of photosynthesis (Fig. 8).

**Net respiration**

The respiratory activity did not show any definite trend like that of net photosynthesis. During May the rate of respiration was found to be high (1.1 ml $O_2$/g DW/ h) in *G. edulis*. The ratio between net photosynthesis and net

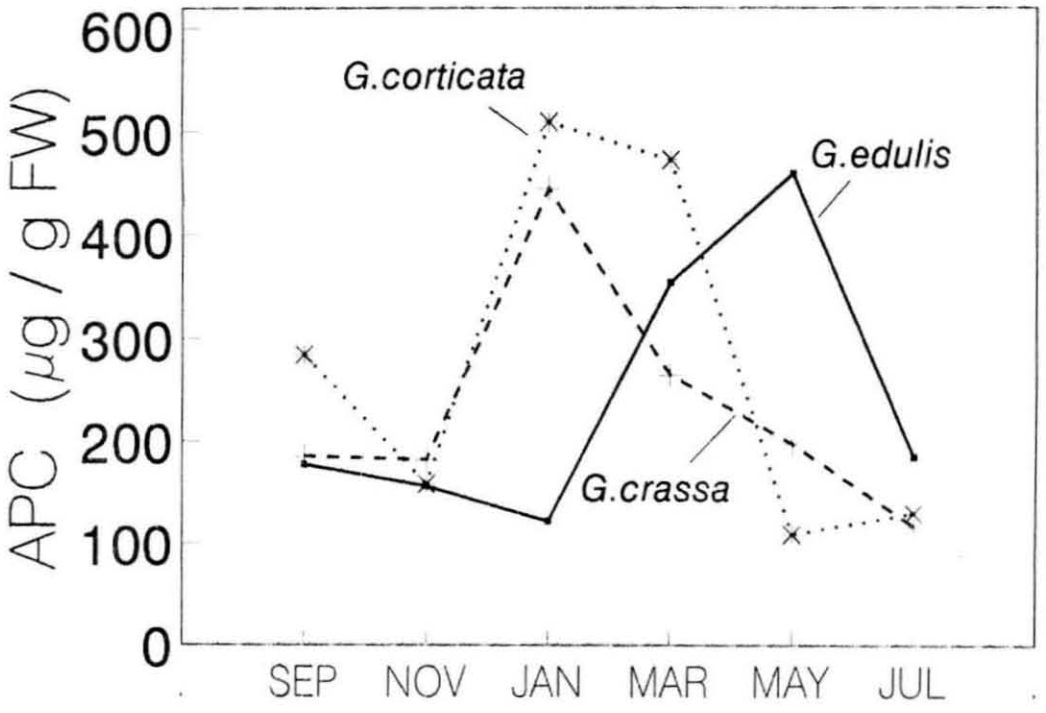


**Fig. 7**

Changes in allophycocyanin content of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. The mean values are expressed in µg/g FW. Values represent average of three samples.

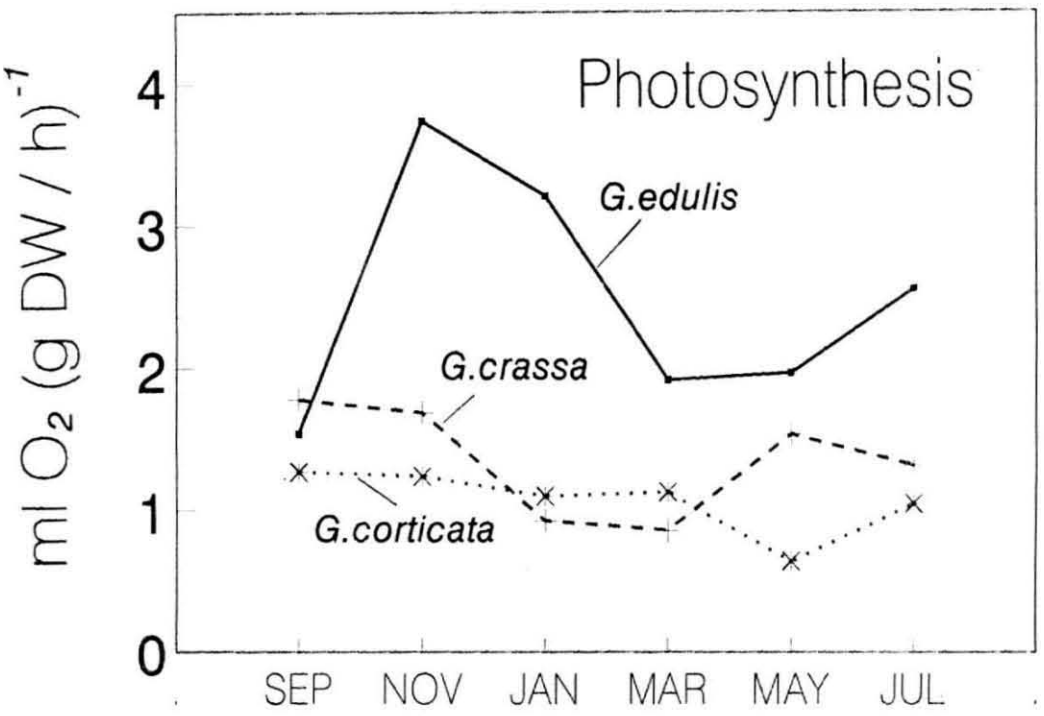


**Fig. 8**

Field photosynthetic activity of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. Photosynthesis was determined by Winkler's method and mean value are expressed in ml/g DW/ h (n=3).

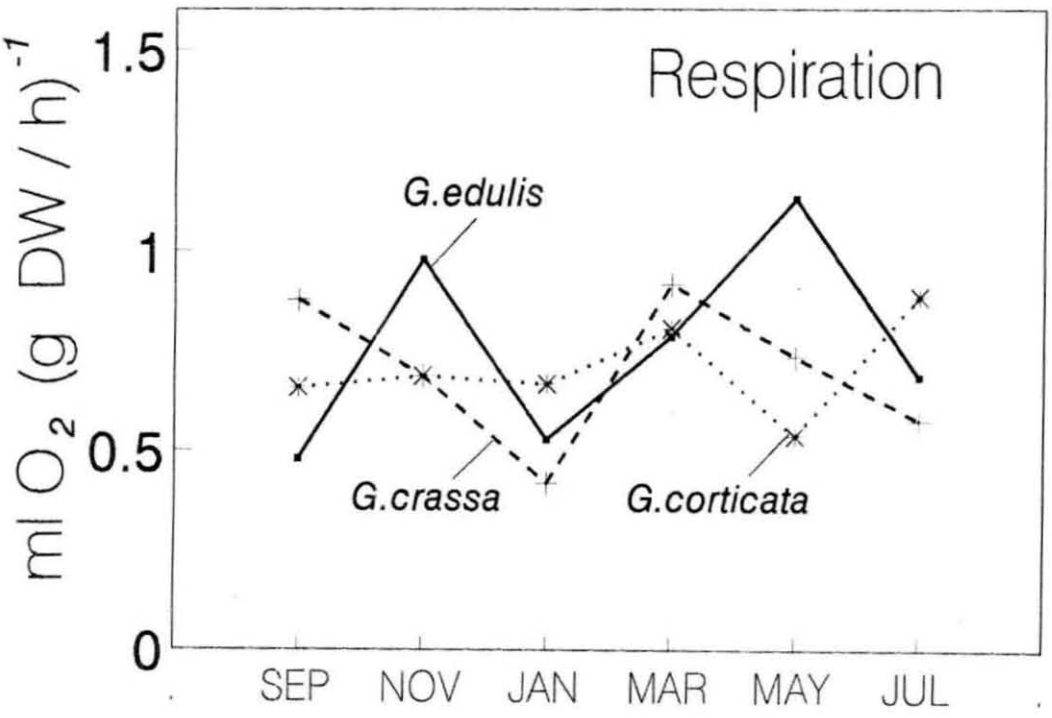


**Fig. 9**

Mean values for the respiratory activity of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. Estimation was made by Winkler's method. Values represent average of three replicates and expressed in ml/g Dw/ h.

respiration showed gradual increase from September to January and then declined till May. It further increased during July corresponding to the growth rate. In *G. crassa*, the net respiration gradually declined from September to January. Maximum respiratory rate was noticed during March (0.9 ml $O_2$/g DW/ h), and then declined till July. In *G. corticata* rate of net respiration gradually increased till March and then declined in July (0.54 ml $O_2$/g DW/ h). Maximum value was noticed during May (0.81 ml $O_2$/g DW/ h). The ratio between net photosynthesis and net respiration was found to be lowest among the three species studied (Fig. 9). It exhibited a gradual decline from November (0.19 ml $O_2$/g DW/ h) to July (0.12 ml $O_2$/g DW/ h).

Photosynthetic activity measured by polarographic method showed variation from the field photosynthetic measurements. In *G. edulis* the photosynthetic activity showed bimodal pattern like growth. It increased from September to January and then declined till May. Further, it exhibited an increase to 10.92 µmol/g FW/ h in July. The photosynthetic activity showed significant positive correlation with growth ($r = 0.95$). *G. crassa* showed a similar trend like *G. edulis*. The photosynthetic activity increased from September to January and then declined till May (3.84 µmol/g FW/ h). Maximum value was noticed during January (9.15 µmol/g FW/ h). It exhibited a significant positive correlation with growth ($r = 0.75$). *G. corticata* showed maximum photosynthetic activity among the three species studied in this experiment. Maximum activity was observed during September (28.57 µmol/g FW/ h) and it declined gradually till January. Further, there was no definite pattern in the activity (Fig. 10). It also showed a positive correlation with growth ($r = 0.81$).

**Agar yield**

Agar content of *Gracilaria* varied among the species and also seasonally. In *G. edulis* it ranged from 20.5 to 39.8%. From the month of September to November, there was a gradual decline in agar content, and then increased

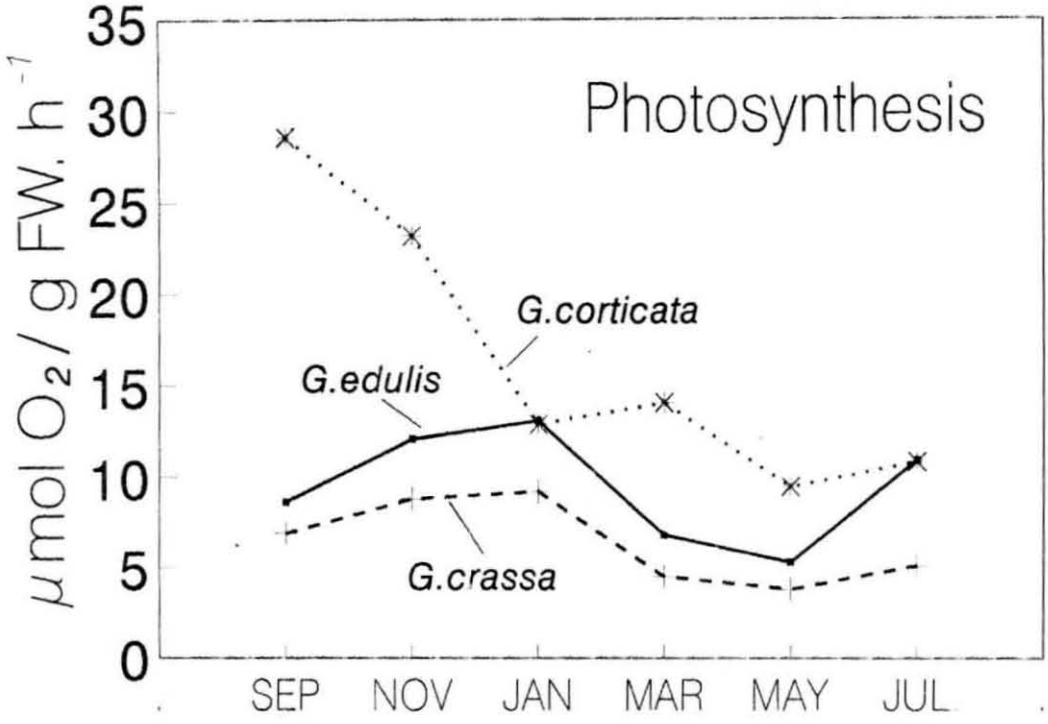


**Fig. 10**

Photosysntshetic acivity of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. Activity was measured under laboratory condition using an oxygen electrode. The average values of three replicates are expressed in umol $O_2$/g FW/ h.

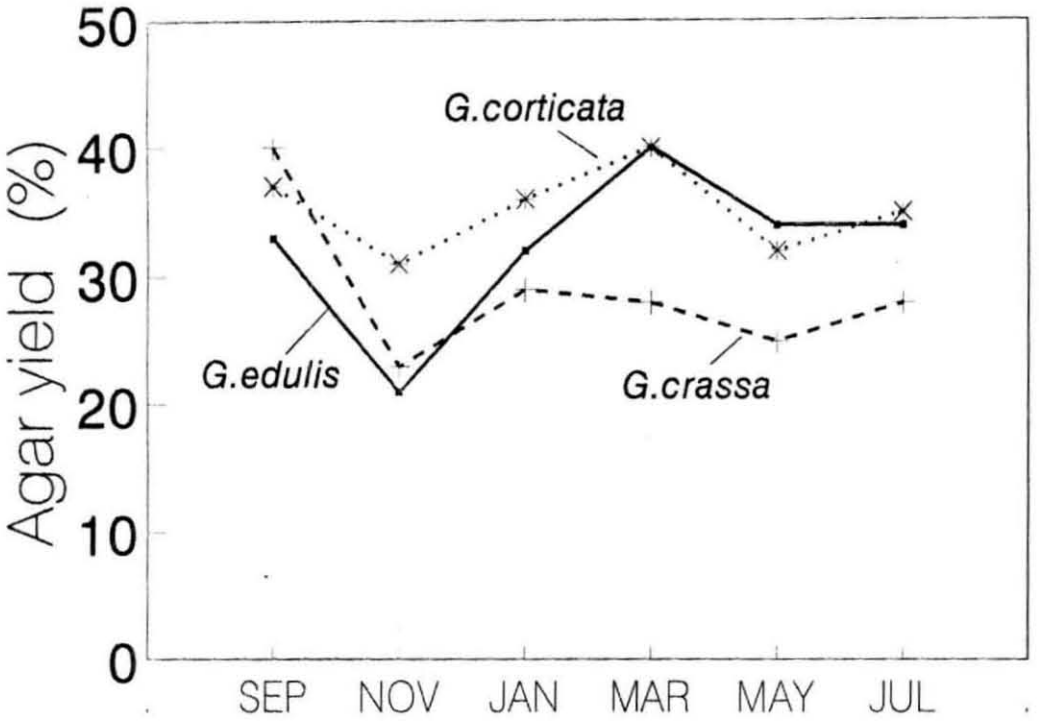


**Fig. 11**

Average values of agar yield extracted from *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. The values are expressed in percentage (n=6).

till March. Further it declined marginally during the lean period of growth. Maximum agar yield was noticed in March (39.79%). During the highest growth period, the agar content was found to be less exhibiting a negative correlation. In *G. crassa*, the agar content ranged from 23.12 to 40.1%. Maximum agar yield was obtained during September and then declined gradually till May with a marginal increase in March. Agar yield showed highly significant positive correlation with biomass (r = 0.96). In *G.corticata* the agar content ranged from 31.1 to 40.0%. Minimum agar yield was obtained during November. There was a gradual increase in agar yield from November to March (Fig. 11). However, there was no marked variation on the agar content throughout the year. It exhibited significant negative correlation with growth (r = -0.75). The higher value of agar yield during September may be due to some experimental error by extraction.

**Gel strength**

The quality of agar can be determined by its properties such as gel strength, gelling temperature and melting temperature. Among the three species of *Gracilaria* studied in the present work, *G.crassa* showed maximum gel strength followed by *G. edulis* and *G. corticata* in that order. It ranged from 28.69 to 59.69 g/cm$^2$ in *G. edulis*. The gel strength of *G. edulis* declined from September to March and then increased. Peak value was obtained in the month of July. Statistically of *G. edulis* did not show any significant relationship with growth and agar yield but a reciprocal relation exist between yield and quality so also with growth. The gel strength of *G. crassa* varied from 40.69 to 77.19 g/cm$^2$. It was maximum during November when the biomass and agar content were high. The gel strength decreased gradually from November to July exhibiting positive relation with growth. In *G. corticata* the gel strength was found to be least among the three species studied. It ranged from 11.05 to 43.69 g/cm$^2$ and showed negative correlation with growth (r = -0.79). There was no definite trend in the variation of the gel strength of *G. corticata* through out the year (Fig. 12).

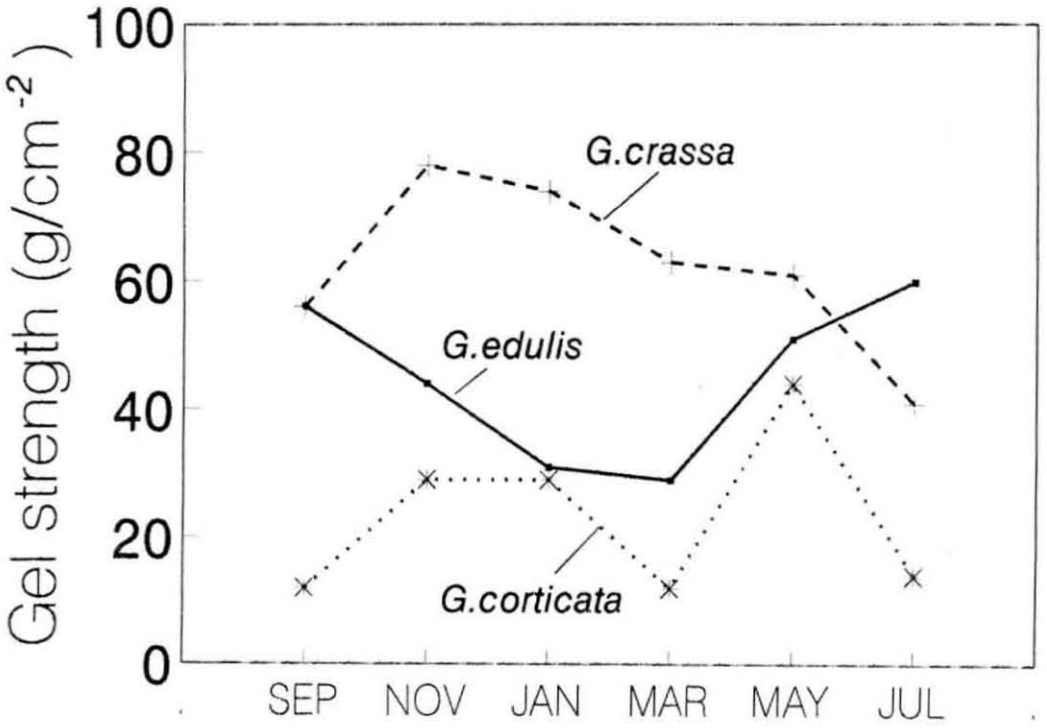


**Fig. 12**

Variations in gel strength of the agar extracted from *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. The average values of three replicates are expressed in gm/cm$^2$.

**Gelling temperature**

There was no marked variation in the gelling temperature among the three species of *Gracilaria* and seasonally within each species. In *G.edulis*, it ranged from 28.0 to 40.0°C. In *G.crassa* the gelling temperature varied between 29.0 and 41.0°C. Gelling temperature of *G. crassa* was maximum among the three species. In *G. corticata* it ranged from 29.5 to 35.5°C. In general, the gelling temperature was low during November and high during September in all the species. In *G. edulis* and *G. crassa* the gelling temperature showed further increase during January and again declined (Fig. 13).

**Melting temperature**

Melting temperature was also found to be maxmimum in *G. crassa*. In *G. edulis* it ranged between 67.0 and 78.5°C but there was no regular pattern in the variation throughout the year. In *G. crassa* the melting temperature ranged between 74.5°C and 89°C. It increased from September to January and then declined till July. Wide range of variation existed between the species in melting temperature of agar. In *G. corticata*, the melting temperature was also found to be least among the species of *Gracilaria* during peak growth period. It ranged from 69.3°C to 77.5°C. Initially the melting temperature declined marginally from September to November and then increased till July with reduction during May (Fig. 14). During lean period of growth the melting temperature of agar was least in *G. edulis*.

**Total protein content**

Total protein content of *G.edulis* increased from September to January and declined there after till the end of May. Further increase was observed during July. Maximum (5.41%) and minimum (3.28%) protein content in *G.edulis* coincided with highest and lowest growth rates during January and

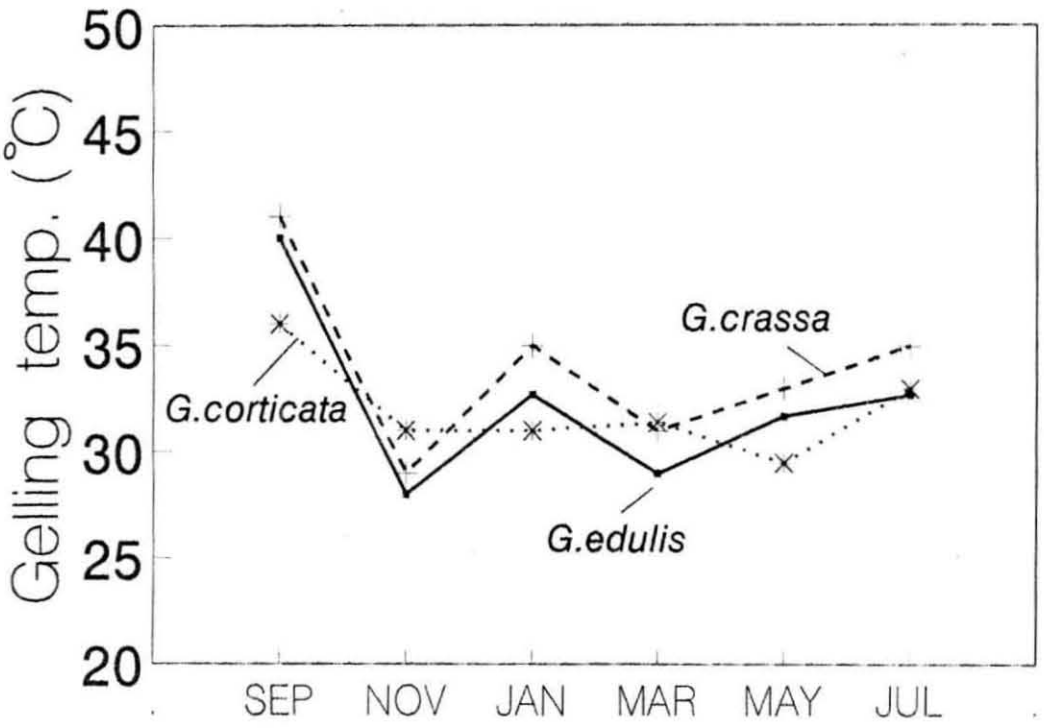


**Fig. 13**

Variations in gelling temperature (in °C) of the agar extracted from *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. The value represent average of three replicates.

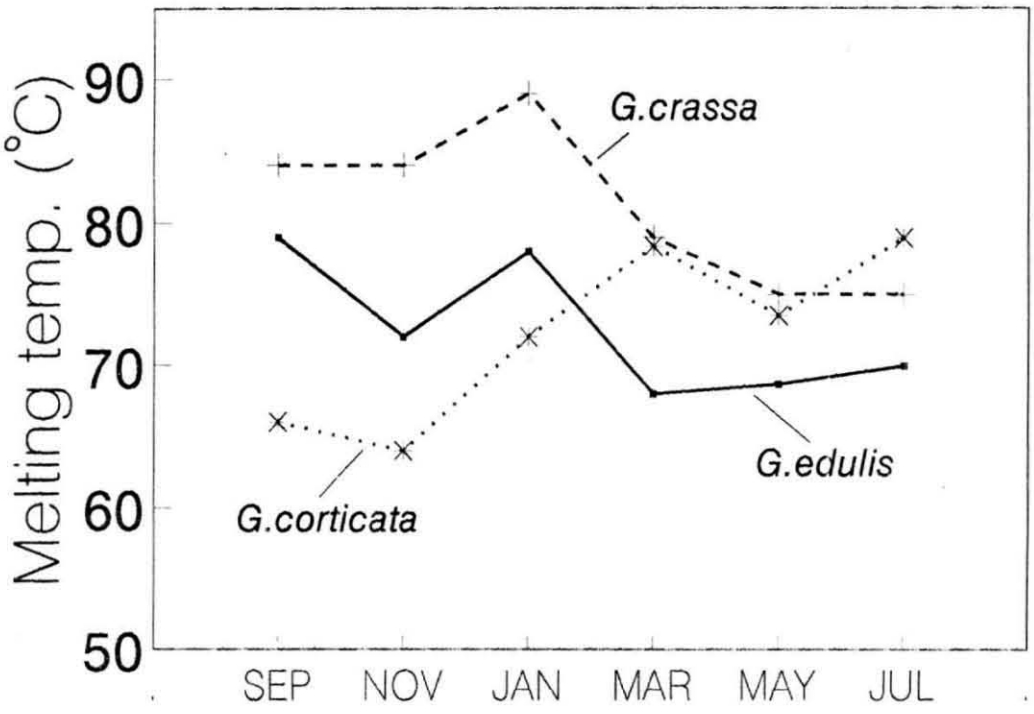


**Fig. 14**

Variations in the melting temperature (in °C) of the agar extracted from *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. The value represent average of three replicates.

May respectively. In *G. crassa* the protein content showed a gradual increase from November (3.13%) to May (5.45%) and then declined. During November, the protein content was found to be lowest. It bore a significantly negative correlation (r = -0.76) with growth. *G. corticata*, exhibited maximum protein content among the three species studied in the present work. It ranged from 4.16 to 9.94%. The protein content increased from September to January and declined during March (Fig. 15). Further increase in May results in indicates a bimodal pattern. It showed a negative correlation with growth (r = -0.83).

**Total carbohydrate content**

Carbohydrate was found to be the major biochemical constituent of *Gracilaria*. In *G. edulis*, the total carbohydrate content was found to be minimum during January (24.2%) when the growth rate was highest and maximum during September (37.68%). Statistically, the carbohydrate content showed a significantly negative correlation with growth (r = -0.89). It also showed a negative correlation with Chl *a* content but positive correlation with agar yield. The carbohydrate content of *G. crassa* gradually declined from September (36.08%) to July (22.10%). Positive correlation was observed between the carbohydrate content and the biomass of this species (r = 0.81). In *G. corticata*, the carbohydrate content was found to be least among the three species of *Gracilaria* studied in this present work. It ranged from 19.75 to 35.35%. It increased gradually from September to May and then declined. The maximum (35.35%) and minimum (19.75%) values of total carbohydrate in *G. corticata* coincided with period of lowest and highest growth in May and September, respectively. This negative relationship was statistically significant (r = -0.95). Total carbohydrate content of *G. corticata* also bore a positive correlation with agar yield (Fig. 16).

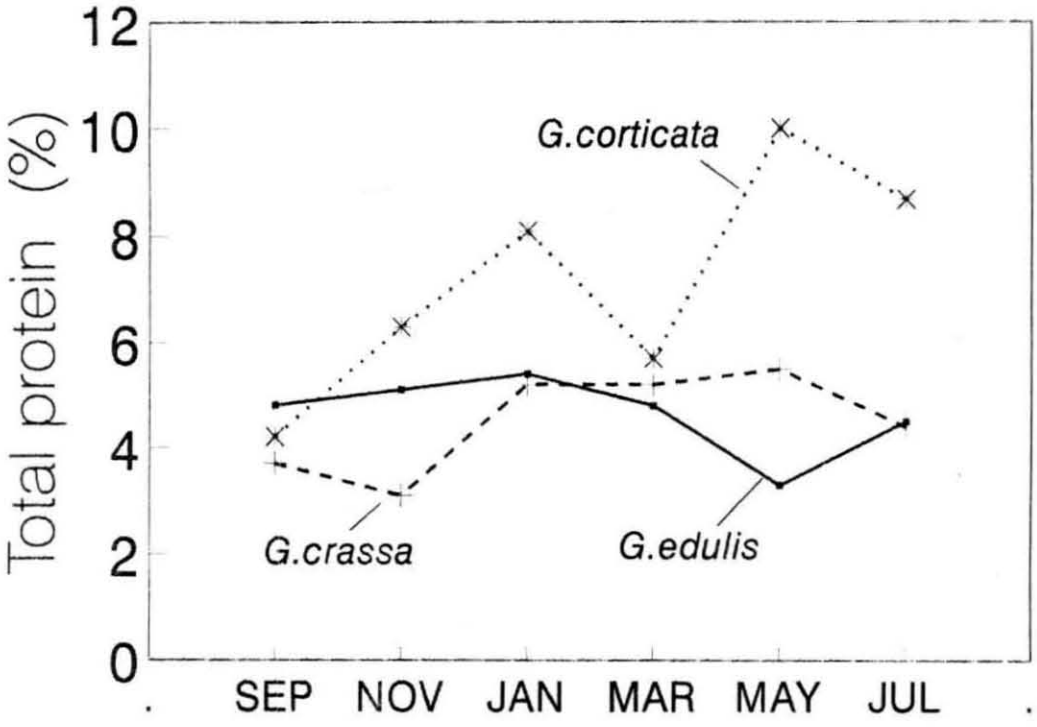


**Fig. 15**

Total protein content of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. For detail on extraction and estimation of protein see Materials and methods.

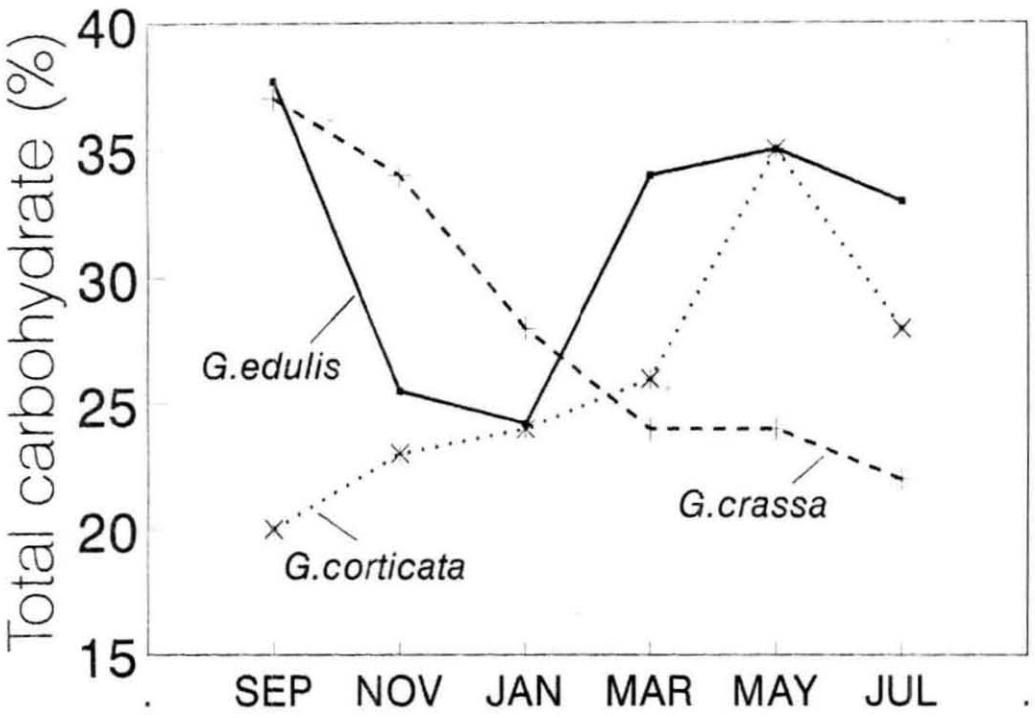


**Fig. 16**

Total carbohydrate content of *Gracilaria edulis, G. crassa* and *G. corticata* collected from Thonithurai and Pudumadam at different periods of the year. Values represent average of three samples. For other details, see Materials and methods.

**Total lipid content**

Among the three major biochemical constituents namely protein, carbohydrate and lipid, the latter showed minimum value in *Gracilaria*. In *G. edulis* it ranged from 0.96 to 3.97%. It increased from November to May and then declined in July. In *G. crassa* the lipid content showed irregular variations throughout the year. It ranged from 0.89 to 3.55%. In this species, the maximum lipid content was found during July. In *G. corticata* lipid content varied between 0.465 and 3.35%. There was a gradual increase from September to May and then declined (Fig. 17). It showed significant negative correlation with growth (r = -0.80).

**Mineral constitutents**

Mineral constituents of *Gracilaria* varied among the species and also seasonally within the species.

**Manganese**

In *G. edulis* maximum concentration of manganese was noticed during March (0.0077%). It declined gradually to reach the trough in January (0.0042%). In *G. crassa* the manganese content ranged from 0.0008 to 0.0061%, maximum was seen during May and declined till November. Further, it increased from January. In *G. corticata* manganese content declined gradually from January till November (Table 1).

**Copper**

In *G. edulis* there was not much variation in the copper content from March to July. However, it declined there after till November. The copper content of *G. crassa* increased gradually from September (0.0025%) to July (0.006%). *G. corticata* did not show any regular pattern of variation through out the year. The copper content ranged from 0.0025 to 0.0037% in this species with a peak during May (Table 1).

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**Table 1**

Seasonal variations on the mineral constitutents (Mn, Pb, Cu) of *Gracilaria* spp.

| Species | September | November | January | March | May | July |
|---|---|---|---|---|---|---|
| **Manganese (%)** | | | | | | |
| *G. edulis* | 0.0063 | 0.0061 | 0.0042 | 0.0077 | 0.0062 | 0.0052 |
| *G. crassa* | 0.0020 | 0.0027 | 0.0035 | 0.0047 | 0.0061 | 0.0029 |
| *G. corticata* | 0.0025 | 0.0025 | 0.0040 | 0.0035 | 0.0038 | 0.0032 |
| **Lead (%)** | | | | | | |
| *G. edulis* | 0.0045 | 0.0040 | 0.0045 | 0.0045 | 0.0065 | 0.0061 |
| *G. crassa* | 0.0033 | 0.0050 | 0.0050 | 0.0066 | 0.0059 | 0.0059 |
| *G. corticata* | 0.0036 | 0.0029 | 0.0043 | 0.0041 | 0.0060 | 0.0061 |
| **Copper (%)** | | | | | | |
| *G. edulis* | 0.0031 | 0.0030 | 0.0032 | 0.0047 | 0.0046 | 0.0047 |
| *G. crassa* | 0.0025 | 0.0031 | 0.0035 | 0.0040 | 0.0042 | 0.0060 |
| *G. corticata* | 0.0027 | 0.0029 | 0.0025 | 0.0027 | 0.0037 | 0.0019 |

**Lead**

Lead content showed a peak value (0.0065%) in May and declined gradually till November (0.004%) showing a negative relationship with growth. Further, it increased from January. Lead content of *G. crassa* was maximum during March (0.0066%) and gradually declined till September (0.0033%). Further it increased from November. In *G. corticata* it ranged from 0.0029 to 0.0061%. The lead content increased gradually from November to July and then declined (Table 1).

**Zinc**

Compared to manganese, copper and lead, zinc was present in relatively higher concentration in *Gracilaria*. In *G. edulis*, zinc content was found to be maximum during January and gradually declined till November. In *G. crassa* it declined from September till to July. *G. corticata* showed a decline in zinc content from the month of September to March and then increased to attain peak value in July (0.0116%). It almost showed a positive correlation with growth (Table 2).

**Magnesium**

Magnesium content of *G. edulis* ranged from 0.88 to 1.21%. The lowest value was observed during September and gradually increased till May. Further, it declined from the month of July. In *G. crassa* the magnesium content ranged betwen 0.86 to 1.58% showing no definite pattern in variation throughout the year. In *G. corticata* the magnesium content was maximum during January (1.32%) and gradually declined till November (Table 2).

**Iron**

*G. edulis* showed maximum iron content in January (0.73%) and gradually declined to reach a trough in November (0.43%). Iron content of *G. crassa* varied in a similar manner like magnesium. It ranged from 0.4 to 0.8% with

**Table 2**

Seasonal variations on the mineral constitutents (Zn, Mg, Fe) of *Gracilaria* spp.

| Species | September | November | January | March | May | July |
|---|---|---|---|---|---|---|
| **Zinc (%)** | | | | | | |
| *G. edulis* | 0.0097 | 0.0072 | 0.0291 | 0.0126 | 0.0101 | 0.0099 |
| *G. crassa* | 0.0115 | 0.0106 | 0.0088 | 0.0088 | 0.0076 | 0.0069 |
| *G. corticata* | 0.0093 | 0.0068 | 0.0056 | 0.0056 | 0.0089 | 0.0116 |
| **Magnesium (%)** | | | | | | |
| *G. edulis* | 0.88 | 0.65 | 1.00 | 1.11 | 1.21 | 1.06 |
| *G. crassa* | 1.45 | 0.86 | 1.02 | 1.58 | 0.88 | 1.23 |
| *G. corticata* | 0.70 | 0.62 | 1.32 | 1.05 | 0.79 | 0.71 |
| **Iron (%)** | | | | | | |
| *G. edulis* | 0.54 | 0.43 | 0.73 | 0.65 | 0.63 | 0.62 |
| *G. crassa* | 0.64 | 0.40 | 0.76 | 0.80 | 0.56 | 0.70 |
| *G. corticata* | 0.43 | 0.37 | 0.45 | 0.43 | 0.55 | 0.43 |

a peak value in March. Iron content of *G. corticata* was found to be maximum during May and declined there after till November. Iron content was found to be more in *G. crassa* followed by *G. edulis* and *G. corticata* (Table 2).

**Calcium**

Among the three species studied in the present work, *G. crassa* had the maximum calcium content. In *G. edulis* it increased gradually from a least value (0.64%) in November to a maximum value (1.59%) in May and then declined showing almost a negative pattern with growth. In *G. crassa* the calcium content ranged from 5.8% in November to 11.04% in March. There was no regular variation in calcium content of *G. crassa* through out the year. In *G. corticata* the calcium content was maximum during January (1.54%) and least during November (0.85%). The calcium content was found to be lowest in *G. corticata* compared to *G. edulis* and *G. crassa*. Here also there was no definite pattern of variation in calcium content throughout the year (Table 3).

**Sodium**

*G. edulis* contained relatively more sodium than *G. corticata* and *G. crassa*. In *G. edulis* sodium content was maximum during January (7.08%) and least during September (1.36%). It increased from the month of September and peaked during January. Further, it did not show any definite pattern. However, during May there was enhancement of sodium content. In *G. crassa* maximum sodium content was obtained during May (7.2%) corresponding to the lean growth period and least during September (1.56%). High sodium content was also found during November. Sodium content of *G. corticata* was maximum during January (6.52%). Higher sodium content was also obtained in September (4.84%). From the month of January to July, there was a gradual decline of sodium content. Further it increased during September (Table 3).

**Table 3**

Seasonal variations on the mineral constitutents (Ca, Na, K) of *Gracilaria* spp.

| Species | September | November | January | March | May | July |
|---|---|---|---|---|---|---|
| **Calcium (%)** | | | | | | |
| *G. edulis* | 0.86 | 0.64 | 1.24 | 1.16 | 1.59 | 1.16 |
| *G. crassa* | 8.12 | 5.80 | 8.52 | 11.04 | 8.80 | 9.28 |
| *G. corticata* | 1.22 | 0.85 | 1.54 | 1.25 | 1.04 | 1.28 |
| **Sodium (%)** | | | | | | |
| *G. edulis* | 1.36 | 1.44 | 7.08 | 2.24 | 3.68 | 2.84 |
| *G. crassa* | 1.56 | 5.92 | 2.68 | 1.68 | 7.20 | 2.92 |
| *G. corticata* | 4.84 | 1.56 | 6.52 | 3.48 | 2.84 | 2.80 |
| **Potassium (%)** | | | | | | |
| *G. edulis* | 3.84 | 6.64 | 2.24 | 2.28 | 3.72 | 2.24 |
| *G. crassa* | 1.32 | 4.48 | 2.08 | 1.44 | 3.96 | 2.40 |
| *G. corticata* | 4.52 | 2.96 | 6.16 | 2.72 | 3.32 | 2.64 |

### Potassium

Potassium was found to be more in *G. edulis*. Its content was maximum during November (6.64%) and minimum during July (2.24%). There was no regularity in the variation of potassium content throughout the year. However, a marginal increase was observed during the lean growth period. In *G. crassa* the potassium content was found to be maximum during November (4.48%) and least during September (1.32%). High potassium content was also observed during May. In *G. corticata* the potassium content ranged from 2.64% in July to 6.16% in January. High potassium content was also noticed during September (4.52%) in this species (Table 3).

In general, the concentrations of mineral constituents in all the species of *Gracilaria* were showing a similar seasonal pattern and an order such as Na > K > Ca > Mg > Fe > Zn > Pb > Mn > Cu.

## Environmental parameters

### Salinity of seawater

The salinity of seawater ranged from 25.96 to 36.93 ppt at Thonithurai and 28.56 to 35.05 ppt at Pudumadam. Lowest salinity was estimated during January and highest during September at both collection sites. The salinity of seawater increased gradually from March to September and then declined till January (Table 4).

### Dissolved oxygen content

Dissolved oxygen content of seawater was found to be maximum during January at Thonithurai (5.85 ml/ l) and lowest during July (2.36 ml/ l). At Pudumadam the dissolved oxygen content was highest during March (5.78 ml/ l) and least during May (4.08 ml/ l). In general, the dissolved oxygen content of seawater at Pudumadam was more compared to Thonithurai. There was no regularity in the variation of dissolved oxygen content Pudumadam throughout the year. Higher dissolved oxygen was obtained during September and January (Table 4).

**Table 4**

Seasonal variations in hydrological parameters at Thonithurai and Pudumadam.

| Months | DO (ml/l) | | Salinity (o/oo) | | SWT (°C) | |
|---|---|---|---|---|---|---|
| | THN | PDM | THN | PDM | THN | PDM |
| September | 5.12 | 5.12 | 36.9 | 35.1 | 27.3 | 29.6 |
| November | 3.95 | 4.35 | 30.2 | 30.9 | 27.6 | 28.6 |
| January | 5.85 | 5.03 | 26.0 | 28.6 | 29.8 | 26.2 |
| March | 2.59 | 5.78 | 27.6 | 29.0 | 27.8 | 27.2 |
| May | 2.63 | 4.08 | 31.1 | 30.9 | 27.8 | 28.4 |
| July | 2.36 | 4.38 | 32.5 | 33.1 | 27.8 | 30.0 |

**Table 5**

Seasonal variation on the metereological parameters collected from Pamban meterological centre.

| Months | Rain fall (mm) | Sunshine (hours) | maximum temp. (°C) | minium temp. (°C) |
|---|---|---|---|---|
| September | 0.8 | 248.8 | 33.3 | 27.0 |
| November | 325.4 | 139.3 | 29.5 | 25.0 |
| January | 132.0 | 219.7 | 29.0 | 24.4 |
| March | Nil | 300.9 | 32.8 | 25.1 |
| May | 88.5 | 240.3 | 33.8 | 27.8 |
| July | trace | 228.1 | 33.5 | 27.3 |

### Surface water temperature

Surface seawater temperature showed a different trend than the atmospheric temperature. At Thonithurai it ranged from 27°C to 29.8°C and at Pudumodam it ranged from 26.2 to 30°C. At Thonithurai there was a gradual increase of surface water temperature from September to January and declined there after but at Pudumadam, the surface water temperature decline from September to January and then increased till July (Table 4).

## Meterological data

### Rain fall

The rain fall was maximum during November (325.4 mm) and gradually declined in January (132 mm). No rainfall was noticed during March. In the month of May, 88.5 mm rainfall was recorded. Further during July and September, the rainfall was very trace (Table 5).

### Sunshine hours

Sunshine hours showed a negative relationship with rainfall. It was maximum during March (300.9 h) when there was no rain at all. The sunshine hours also found to be more during September (248.8 h) and May (240.3 h). Least value was noticed during November (139.3) corresponding to highest rainfall (Table 5).

### Maximum temperature

Maximum temperatures of the atmosphere (average of one month) showed a peak value during May. It gradually declined from May to January followed by an increase from the month March (Table 5).

### Minimum temperature

Minimum atmospheric temperature ranged between 24.4 to 27.8°C showing the peak value during May and least during January. It showed a similar trend like the maximum temperature (Table 5).

# Chapter I

# Discussion

Considerable morphological adaptation was found in *Gracilaria edulis*, *G. crassa* and *G. corticata* in their natural habitat vis-a vis changes in environmental conditions. During peak growth season, *G. edulis* and *G.crassa* were bright green in colour and exhibited increased branching pattern. During lean growth season, the species of *Gracilaria* changed the color to dull brown particularly during March and May at high PFD and high temperature. Besides, the axis becomes thick and less branched. The percentage of dry weight was found to be higher during the lag period of growth. Similar observations were made by Lapointe (1981). The dry weight percentage of *Gracilaria* ranged between 10 and 20% of fresh weight.

Increased productivity is usually correlated with vigorous water motion, ensuring availability of nutrients as well as uniform conditions of temperature and salinity. However, extreme turbulance caused by storms would reduce biomass of seaweeds. All the three species of *Gracilaria* are available in the Gulf of Mannar in South-east coast of Tamilnadu. This part of the sea is usually turbulent from April to September due to effect of South-west monsoon, thus reducing the biomass per unit area. In the Gulf of Mannar, *Gracilaria edulis* and *G. crassa* occur more abundantly in calmer areas of sea including island waters. These species are relatively slender and prone to breakage by strong wave action. *G. corticata* is more robust, cartilagenous and prefers to grow on rocks and sand in the near coast, either firmly attached to the rocks by holdfast or buried in the sand and always exposed to extreme wave actions. The decline of seaweed biomass during May, was not due to the effect of sea conditions but probably due to excessive temperature and light intensities.

The shallow water habitat of *Gracilaria* species is subjected to seasonal variations in temperature and light intensities. During summer, the temperature even exceedes 33°C. With increasing temperature, productivity may be reduced

or even cease as shown by Li *et al.* (1984) and Wang *et al.* (1984a). The combined effect of high light intensity and extreme temperature often results in lowering the photosynthetic rate as observed in the present study. During the month of May, photosynthetic activity of *Gracilaria* species has reduced to a marked extent. Whereas it increased during July, when the temperature was still high but light intensity and total sunshine hour reduced. This suggests that the enhanced temperature tolerance at moderate light could be due to the fact that these plants are able to maintain a pH gradient and activated status of the photosynthetic apparatus which require only low level of light as observed by Heldt *et al.* (1983). Photosynthetic electron transport is cited as the most thermally labile aspect of the photosynthetic apparatus where photoinhibitory damage is most evident (Powles 1984, Chetti and Nobel 1987). Havaux (1991) found that PSII was inhibited and PSI stimulated within minutes after transfer to high temperature in peas. The pattern of photosynthetic response to high temperature is similar to those reported in other red algae (Mathieson and Burns 1971, Laponite *et al.* 1984, Smith and Berry 1986, Luning 1990).

Overall, the present results are in agreement with those of other workers (Bolton 1983, Gerard and Dubois 1988) and support the hypothesis that temperature adaptation of photosynthesis is important in controlling seaweed growth. Both high and low temperature enhanced photoinhibition have been observed in unicellular algae and higher plants (Sadakane *et al.* 1981, Ludlow 1987, Oquist *et al.* 1987). In *Porphyra* it was suggested that the mechanism of photoinhibition is similar as that in higher plants (Bose *et al.* 1988, Herbert and Waaland 1988).

Photoinhibition is a reversible reduction process in quantum yield of photosynthesis, related to absorption of light energy in excess which produces chlorophyll bleaching (Osmond 1981). Seasonal variation in chlorophyll content showed that all the species of *Gracilaria* exhibited a reduction in chlorophyll

content during the month of May when the light intensity and sunshine hours were found to be very high. Greer *et al.* (1986) opined that reduction in photosynthesis on high PPFD may result in damage of photosynthetic apparatus but the importance of light on photosynthesis and production is a difficult parameter to assess. However, in the present study, all the three species of *Gracilaria* exhibited a strong positive correlation of photosynthesis and growth. There is little evidence that *G edulis* and *G. crassa* where as *G. corticata* are more tolerant to high radiation are sensitive to high level of radiation. Generally, best growth occurs at or near the surface of water at high irradiance and production rate declined with increasing depth. Dawes *et al.* (1978) explained that *Gracilaria* sp. was found to require low photon fluence rates for optimal growth and photosynthesis and show low light compensation point. However, *G. verrucosa* was found to have higher light saturation points (about 600 $\mu E\ m^{-2}.S^{-1}$) than other algal species. Lapointe (1981) also found such high optimal light requirements for *G. tikvahiae* and suggested that light might be the environmental factor limiting growth. But variation in response to light, within the same species at various season, growth habitat and even along the same thallus was reported (Mathieson and Norall 1975a,b, Dawes 1978, Durako and Dawes 1980, Gleen and Doty 1981). In the present study, it was observed that there was a marked variation among the species of *Gracilaria* to utilize the light energy. *G. corticata* can utilise high light intensities approaching to full sunlight compared to the other two submerged species, *G. edulis* and *G. crassa* which are found to utilze low lights intensity efficiently.

As a secondary effect of light radiation desiccation may be a serious problem when the thalli become exposed and surface water temperature increased. It was shown that *Gracilaria* can maintain net production under low light (Rosenberg and Ramus 1981, Lapointe *et al.* 1984). This may be the reason that some species can even thrive in turbid habitat with low illumination.

Submerged plants are subjected to sharply decreasing light intensities along the depth gradient. Such plants readily respond to low light condition by increasing pigment content (Steeman and Nielsen 1975, Senger and Fleischhacker 1978). Similar observations were made in green (Yokohama and Kageyama 1977), brown (Wheeler 1980) and red (waaland *et al.* 1974, Rhee and Briggs 1977, Lapointe 1981) algae. In the present study, active photosynthesis was observed at low sunshine hours and low temperature during November-January when the chlorophyll content was found to be higher. The chlorophyll content of *Gracilaria* species showed positive correlation with photosynthetic activity.

Beer and Levy (1983) concluded based on their studies that plants grown at low PFD appeared dark red while the one growing in high light appeared to be greenish brown. But this statement appears to be contradictory to the present findings. *G. corticata* growing under high light intensity exposed to full sunlight are bright red in colour than the submerged species of *G. edulis* and *G. crassa* which were found to be greenish brown in colour. The pigment content was also found to be higher in *G. corticata* than in *G. edulis* and *G. crassa*. Thus it may be explained that the colour of the thallus changes in relation to Chl/PE ratio rather than light intensity as observed by Brody and Brody (1962) and Rhee and Briggs (1977). Light intensity may reflect on the changes in pigment to a certain extent based on the location of pigment in chloroplast.

Dring (1981) examined the ecological significance of light adaptation in red algae. He observed changes in pigment composition in red algae with increasing depth. In the present study, it was observed that *G. corticata* was exposed to more light intensity and the chlorophyll and accessory pigments were found to be higher compared to other species. It is understood that there exists wide seasonal variations on the pigment composition of *Gracilaria*, exhibiting higher content during peak growth period. Nonethless, changes in

chlorophyll content were species specific. In *G. edulis*, the accessory pigments were found to be higher when the chlorophyll content was less during the month of May. *G. crassa* did not show marked variation in the pigment composition throughout the year. Though thriving in the same habitats, *G. crassa* and *G. edulis* exhibited wide variation in their pigment content presumably due to difference in their morphology. The present results suggest that seaweeds maximize their photosynthetic capacity by optimizing pigment levels. It was demonstrated that red algae are not phylogenetically better adapted to ambient light than green algae. He pointed out that adaptation to decreasing light intensities was not to maximize absorption of light but rather to optimize light capture in relation to energy requirements for growth.

Salinity was found to be another factor critical for growth and productivity of *Gracilaria*. Excessive rainfall during November reduced the salinity drastically to 27.61 and 28.56 ppt at Thonithurai and Pudumadam, from the high values of 32.54 and 33.12 ppt, respectively during July. Intertidal seaweeds are subjected to salinity stress when exposed at low tide or trapped in tide pools. When fresh water from rain or stream flows, the salinity is lowered while evaporation increases the salinity (Macler 1988). In red algae, altered salinity yield changes in turgor. In *Porphyra purpurea* turgor increases with decreasing salinity (Reed *et al.* 1980a). Photosynthesis and respiration have also been shown to be affected by salinity changes. The present results indicate that *G. edulis* exhibits maximum photosynthetic activity and growth at a salinity range of 26-28 ppt. Salinity effect on plant growth is highly species specific. *G. corticata* was found to grow better even under higher salinity unlike *G. edulis* but because of open sea impact, the effect of uniformity of temperature and salinity exists in this area. Seaweeds can be classified by their response to salinity. In the present study, *G. corticata* was found to be on euryhaline, *G. edulis* and *G. crassa* were found to be stenohaline species.

Present investigation indicates that, agar content has negatively correlated with the growth in *G. edulis* and *G. corticata*, while *G. crassa* showed a positive correlation. According to Lahaya *et al.* (1986), high growth rate under optimal condition gives a high yield of extractable material but low yield of autoclavable fraction. The biomass from the fast growing strains consists of young tissue while slow growing plants have a high content of mature tissue. Thus composition of agar reflects the average age of plant materials. The influence of tissue age on agar quality was discussed by Bird *et al.* (1981) and Craigie and Wen (1984) . Similar observations were made earlier (Hoyle 1978, Oza 1978) that agar content was relatively low when the growth rate was high. Seasonal variations do not affect the agar content in *G. bursapastoris* to the same extent as they do in *G. verrucosa* (Whyte *et al.* 1981). *G. crassa* showed similar results. Marginal variation in the yield may be due to some experimental error. The effect of natural environmental conditions on the seasonal variations in the production of cell wall polysaccharides (agar or carrageenan) are reported (Kim and Haumm 1965, Oza 1978, Asare 1980).

In the present work, the quality of agar did not show any significant relationship with growth in *G. edulis* and *G. crassa*, but *G. corticata* exhibited a negative correlation. In general, the gel strength declined in mature tissue when the yield was high. It was earlier shown that the quality of gel is highly dependent on environmental factors (Christiaen *et al.* 1987, Bird 1988). It is also mentioned that the composition of agar during winter is of high quality, when the thalli are highly branched with short fronds. Doty and Santos (1983) explained that, the sulfate content of *Gracilaria* depends on both environmental condition and the species.

The biochemical constitutents of *Gracilaria* showed reciprocal relationship with growth which indicates the utilization of these materials for growth. *G. crassa* only exhibited a positive correlation of total carbohydrate, which is an

index of agar yield with growth. The accumualtion of these essential biochemical constitutents was observed during lag period of growth.

Metals are classified into three catagories: Non-critical or toxic, very toxic and relatively accessible (Wood 1974). Some of the heavy metals such as Fe, Cu, Zn and Mn are essential micro nutrients and known as trace metals. Marine algae concentrate the metal ions from seawater and variation in the concentration of the metals in the thallus is often taken to reflect metal concentration of surrounding seawater. Therefore, it acts as an indicator of trace metals. Present results show that metals such as Ca, Na, Pb, Mn, Cu, Fe and Mg accumulate in the seaweed thallus during lag phase of growth. This observation is in agreement with that of Bryan and Hummerstone (1973). They also have found that metal accumulation was influenced by the position of algae on the shore and the season of the year (Burdon-Jones *et al.* 1982).

The order of heavy metal toxicity to marine algae was studied by Rice *et al.* (1973) and Rai *et al.* (1981). Though copper is an essential micronutrient, it is the second most toxic metal for marine algae (Sunder and Guillard 1976). In the present work, the copper content was found to be high during the lag period of growth when the chlorophyll content was less. The photosynthetic activity was observed to be less with enhanced rate of respiration during that period. It may be explained that, copper toxicity might have some influence to retard growth.

Zinc is generally considered to be actively taken up by seaweeds (Skipnes *et al.* 1975). It was having least toxic effect on the plant. In the present study, zinc was found to be present in a high concentration during the peak growth period in all the species of *Gracilaria*. It is an activator of several important dehydrogenase and protein synthetic enzymes (O'Kelley 1974). An optimum concentration of 0.5 mM of zinc was reported to be required by *Porphyra tenera* without which chlorophyll and phycobilin production was

hindered, and concentration of high molecular weight-protein decreased (Noda and Hariguch 1977). In the present study higher in concentration during peak growth period confirm the above finding.

Manganese plays a vital role in oxygen evolving system of photosynthesis, and is a cofactor for several Kreb cycle enzymes (Bidwell 1979). In the present experiment manganese concentration did not vary much throughout the year. Nonethless, its concentration was high during the lean period of growth.

Photosynthetic and respiratory activities are the most important metabolic processes of plants which ultimately reflect the growth and productivity. Data presented in this work indicates that photosynthetic activity of *Gracilaria* has a positive correlation with growth and the activity was maximum during the period of September-January. This period was marked by low salinity, less sunshine hours, high rainfall, high dissolved oxygen content of sea water and minimum temperature. All these parameters have a cumulative effect on algal physiology and finally on the growth.

# Chapter II

# Results

In India *Gracilaria* species hold great potential for mariculture. Due to depletion of natural stock and over harvesting necessitates to cultivate *Gracilaria* in a large scale. The ecophysiological parameters on biomass, yield, pigment constitutents had a cumulative effect on growth and productivity. These parameters are strongly affected by seasonal changes. The major environmental factors affecting seaweed growth are light, temperature and salinity.

Keeping this in mind, the species of *Gracilaria* were subjected to different treatments of light intensity, spectral quality and salinities to find out the optimum condition for better growth and high productivity.

**Effect of light intensity on photosynthesis**

The light saturation curves for the three species of *Gracilaria* are shown in Fig. 18. In all the species the photosynthetic oxygen evolution reach saturation in the range of 5-10 $W.m^2$. However in *G. crassa* light saturation occurs at lower intensity followed by *G. edulis* and *G. corticata*. In all the photosynthetic measurements, an intensity of 20 $W.m^2$ was employed but saturation obtained within 10 $W.m^2$ light intensities. The photosynthetic activity was found to be maximum in *G. corticata* among the three species of *Gracilaria*. Similar observations were confirmed by $^{14}CO_2$ uptake where the carbon fixed per unit fresh weight was maximum in *G. corticata* (2.13 mmole/ g FW/ h) as compared to *G. edulis* (18.1 mmole/g FW/ h) and *G. crassa* (17.9 mmole/g FW/ h).

**Qualitative studies of lipid**

Qualitative and quantitative variation in fatty acids were observed among the species of *Gracilaria*. The major fatty acids were palmitic acid, palmitoleic acid, myristic acid and myristoleic acid. Besides these, minor quantities of stearic acid and lauric acid were also recorded (Table 23). In *G. edulis*, the lauric acid present in little higher quantity compared to *G. corticata* and *G. crassa*. In *G. crassa* all the major fatty acids were found in comparatively

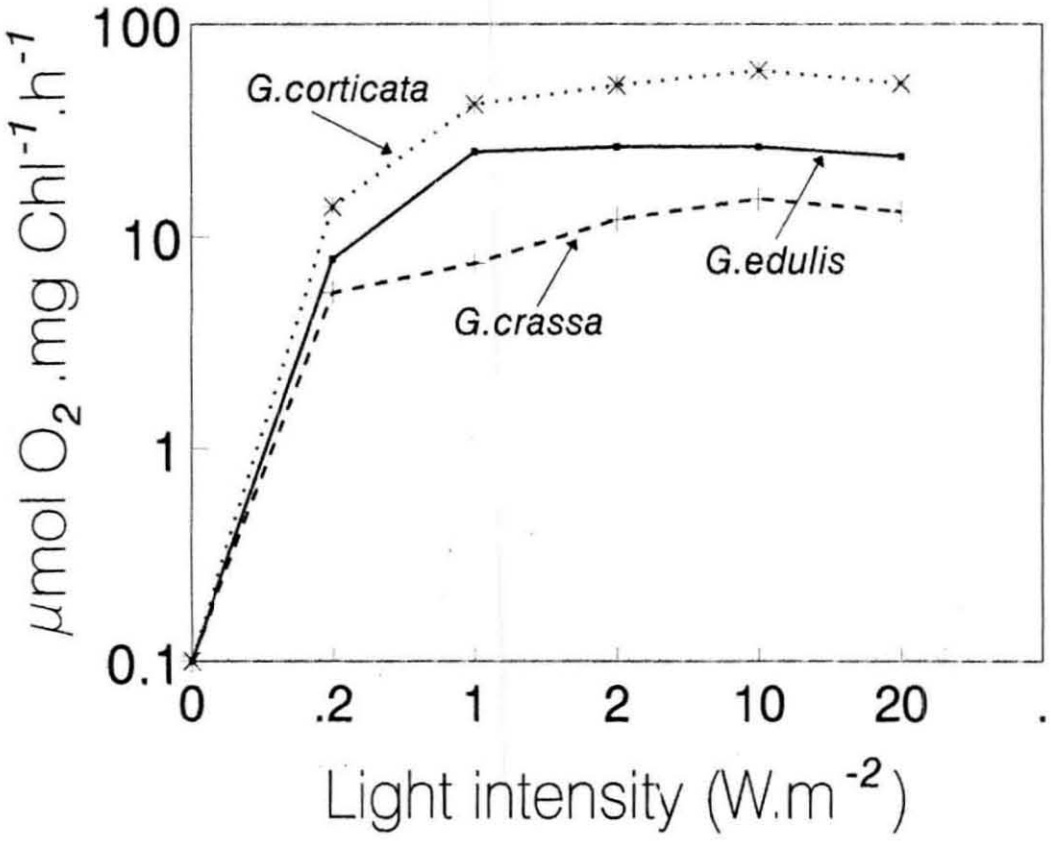


**Fig. 18**

Changes in photosynthetic activity of *Gracilaria edulis, G. crassa* and *G. corticata* as a function of actinic light intensity. Light intensity was varied by using calibrated Schott neutral density filters. Values represent average of three replicates expressed in µmol/g FW/ h.

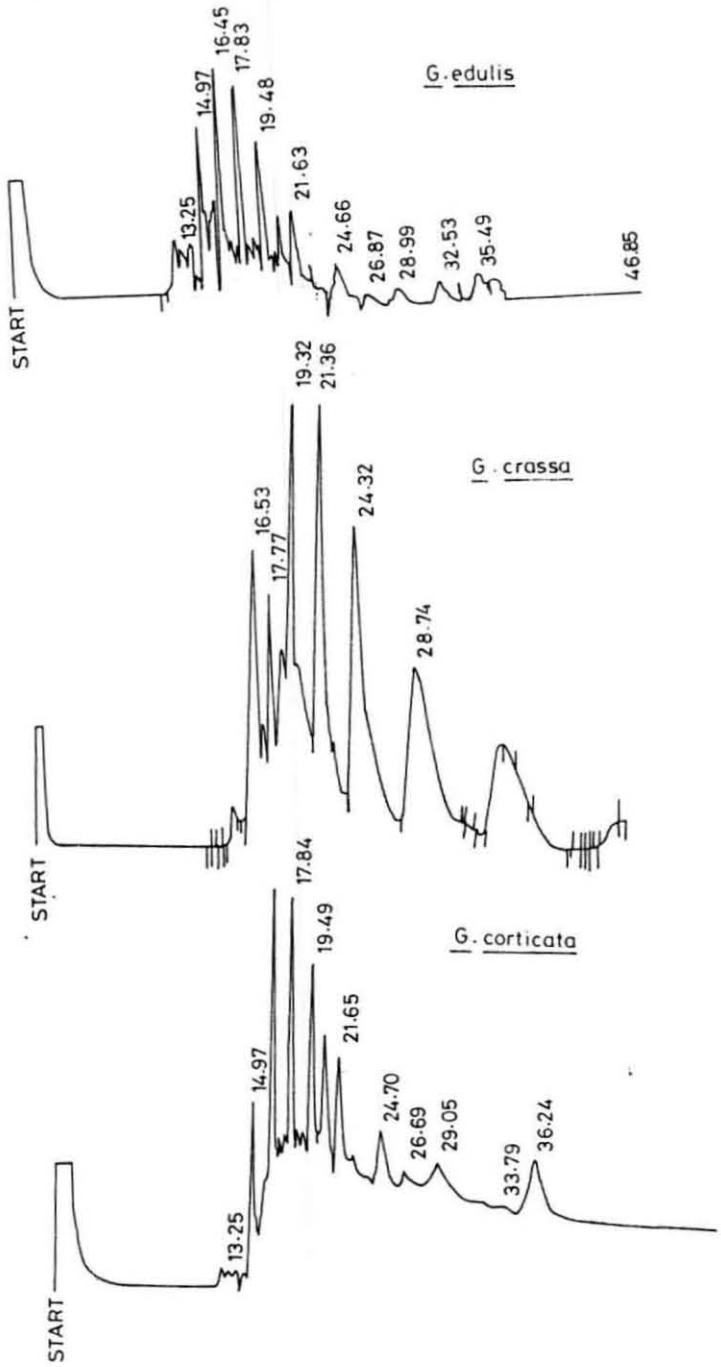


**Fig. 19**

Fatty acids profiles of *Gracilaria edulis, G. crassa* and *G. corticata*. Fatty acids were separated by using HP 5890 gas chromatograph. For other details, see Materials and methods.

lower quantity than in other two species. Stearic acid was only present in *G. edulis* and *G. corticata* but not recorded in *G. crassa*. Among all the fatty acids, myristic acid present in higher quantity than other fatty acids in all the species of *Gracilaria* (Fig. 19).

**Effect of light intensity**

The physiological status of different species of *Gracilaria* was found to be affected when exposed to different light intensities for a period of 12 days.

The Chl *a* content of *G. edulis* declined to 32.6% of initial level, when kept in the high light (HL) compared to 17.3% and 2.7% decrease in intermediate light (IL) and low light (LL) intensities, respectively. Further incubation at different light intensities was found to produce varied responses. On 12th day, it was noticed that the Chl *a* content increased by 87.8% over 6th day level under HL, remain unchanged in IL but further decreased in LL. In *G. crassa*, the Chl content increased more in LL compared to HL and IL intensities on the 6th day of exposure and declined thereafter. Such decline was maximum in HL than in IL and LL. In *G. corticata*, the Chl content increased at all light intensities up to 6th day. Such increase was in the range of 30-38%. Further incubation however decreased the Chl level at all light intensites. The decline was more pronounced under LL than under HL and IL (Table 6).

PE content was found to increase in all the three species of *Gracilaria* under different light intensities during the first 6 days. *G. corticata* exhibited marginal increase of PE content compared to other two species. After 12 days of treatement there was some variation noticed in different species. In *G. edulis*, the PE content declined by 64% in HL but under LL it continued to increase further. In *G. crassa*, treated with low light, showed an increase of PE content over the 6th day value but in IL and HL it declined by 5.6% and 25.8%, respectively. *G. corticata* exhibited a decline in PE content under all treatments. The decline was more pronounced in HL compared to LL and IL intensities (Table 7).

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**Table 6**

Effect of differnt light intensities on chlorophyll content of *Gracilaria* spp.

| Species | Treatment | Chlorophyll content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | HL | 0.0982 | 0.0613 | 0.1151 | -37.58 | +87.8 |
| | IL | 0.0982 | 0.0837 | 0.0845 | -17.32 | + 0.9 |
| | LL | 0.0982 | 0.0956 | 0.0776 | - 2.65 | -19.0 |
| *G. crassa* | HL | 0.0333 | 0.0641 | 0.0262 | +92.58 | -59.1 |
| | IL | 0.0333 | 0.0581 | 0.0448 | +74.52 | -22.9 |
| | LL | 0.0333 | 0.0789 | 0.0713 | +136.9 | - 9.6 |
| *G.corticata* | HL | 0.1161 | 0.1508 | 0.1075 | +29.9 | -28.7 |
| | IL | 0.1161 | 0.1595 | 0.1382 | +37.4 | -13.4 |
| | LL | 0.1161 | 0.1516 | 0.0938 | +30.6 | -38.2 |

**Table 7**

Effect of differnt light intensities on phycoerythrin content of *Gracilaria* spp.

| Species | Treatment | Phycoerythrin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | HL | 0.1278 | 0.2087 | 0.1699 | +63.33 | -18.6 |
| | IL | 0.1278 | 0.1806 | 0.2929 | +41.3 | +62.2 |
| | LL | 0.1278 | 0.2112 | 0.2273 | +65.3 | + 7.6 |
| *G. crassa* | HL | 0.1265 | 0.1858 | 0.1378 | +46.9 | -25.8 |
| | IL | 0.1265 | 0.2058 | 0.1943 | +62.7 | - 5.6 |
| | LL | 0.1265 | 0.1791 | 0.2658 | +41.6 | +48.4 |
| *G.corticata* | HL | 0.3122 | 0.3345 | 0.1578 | + 7.1 | -52.8 |
| | IL | 0.3122 | 0.3609 | 0.2005 | +15.6 | -44.4 |
| | LL | 0.3122 | 0.3719 | 0.1993 | +19.1 | -46.4 |

PC content of *G. edulis* showed maximum increase under HL compared to IL and LL treatment on 6th day of exposure. *G. crassa* also exhibited a similar increase in PC content where as in *G. corticata*, it declined gradually from 0 to 12 days of treatment. In *G. crassa*, PC content declined by 34% in HL but increase under IL and LL intensities (Table 8).

APC content increased in all the species of *Gracilaria* under different light treatment during the first 6 days. Maximum increase of APC was observed under HL in *G. edulis*. *G. crassa* showed an enhancement of APC content by 182.8% under LL. Further treatment up to 12 days, declined the APC content in all light conditions except an increase of 82% in *G. edulis* under IL. *G. corticata* showed maximum decline of APC content among the three species studied (Table 9).

The photosynthetic activity of *Gracilaria* showed variation with respect to treatment under different light levels. The maximum photosynthetic activity in different species varied drastically between 8 to 95 µmol/g FW/ h. Upon treatment to different light intensities for 6 days, the activity declined in *G. edulis* and *G. corticata* but increased in *G. crassa.* Maximum reduction was observed in LL. In *G. crassa* the photosynthetic activity exhibited an increase of 139% under HL compared to IL (120.3%) and LL (5.1% intensities. Further, when the plants were exposed continuously for 12 days to varying light intensities, all the species of *Gracilaria* showed decline in their photosynthetic activity except a recovery in *G. edulis* under LL. In *G. corticata*, no photosynthetic oxygen evolution could be observed in all the treated samples, on the other hand an oxygen uptake reaction was noticed (Table 10).

Absorption spectrum of the thallus of *Gracilaria* species on 0, 6th and 12th day of treatment showed absorption maxima at 676, 621, 565, 495 and 433 nm representing Chl, PE, PC, APC and carotenoid, respectively (Fig. 20). In *G. edulis* the absorption peak at 621, 565 and 495 nm were found to

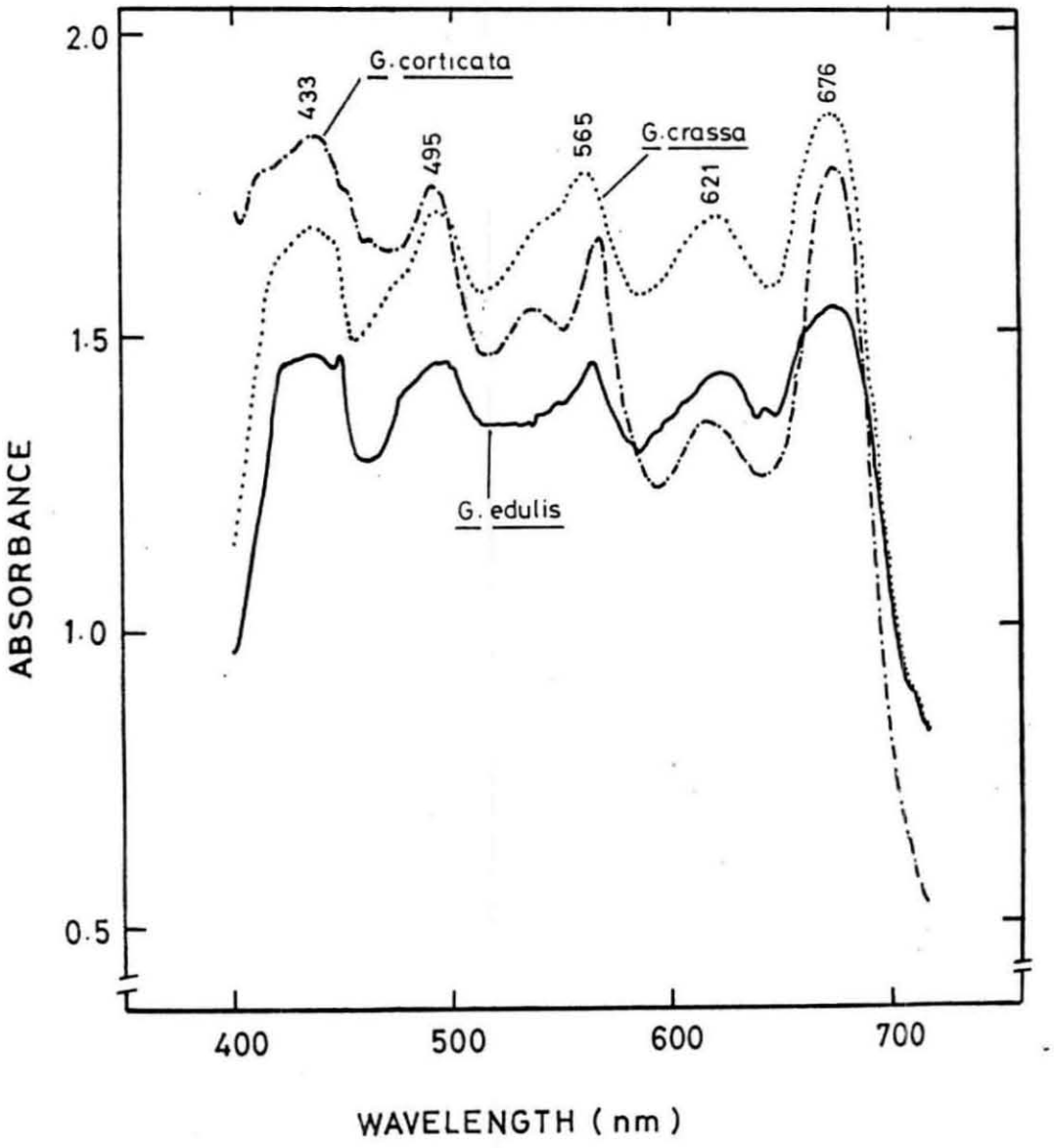


**Fig. 20**

Room temperature absorption spectra of *Gracilaria edulis, G. crassa* and *G. corticata*. For details of spectral measurements, see Materials and methods.

**Table 8**

Effect of differnt light intensities on phycocyanin content of *Gracilaria* spp.

| Species | Treatment | Phycocyanin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | HL | 0.0501 | 0.0799 | 0.0628 | +58.5 | -21.4 |
| | IL | 0.0501 | 0.0781 | 0.1976 | +55.9 | +153.0 |
| | LL | 0.0501 | 0.0703 | 0.0956 | +40.3 | +35.9 |
| *G. crassa* | HL | 0.0600 | 0.0780 | 0.0515 | +30.0 | -33.9 |
| | IL | 0.0600 | 0.0710 | 0.1264 | +18.3 | +78.0 |
| | LL | 0.0600 | 0.0741 | 0.1560 | +23.5 | +110.5 |
| *G.corticata* | HL | 0.1932 | 0.1791 | 0.0558 | - 7.3 | -68.8 |
| | IL | 0.1932 | 0.1645 | 0.0897 | -14.9 | -45.5 |
| | LL | 0.1932 | 0.0786 | 0.0675 | -59.3 | -14.1 |

**Table 9**

Effect of differnt light intensities on allophycocyanin content of *Gracilaria* spp.

| Species | Treatment | Allophycocyanin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | HL | 0.0473 | 0.0791 | 0.0622 | +67.2 | -21.4 |
| | IL | 0.0473 | 0.0526 | 0.0958 | +11.2 | +82.1 |
| | LL | 0.0473 | 0.0743 | 0.0542 | +57.1 | -27.1 |
| *G. crassa* | HL | 0.0404 | 0.1250 | 0.0629 | +209.4 | -49.7 |
| | IL | 0.0404 | 0.1077 | 0.1048 | +166.6 | - 2.6 |
| | LL | 0.0404 | 0.1936 | 0.1024 | +379.2 | -47.1 |
| *G.corticata* | HL | 0.1300 | 0.2123 | 0.0915 | +63.3 | -56.9 |
| | IL | 0.1300 | 0.2674 | 0.0604 | +105.7 | -77.4 |
| | LL | 0.1300 | 0.3676 | 0.0782 | +182.8 | -78.7 |

**Table 10**

Effect of differnt light intensities on photosynthetic activityof *Gracilaria* spp.

| Species | Treatment | Photosynthetic activity (μM/g Fw/ h) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | HL | 95.44 | 64.76 | 11.70 | -32.2 | -81.9 |
| | IL | 95.44 | 47.33 | 11.83 | -50.4 | -75.0 |
| | LL | 95.44 | 4.61 | 8.31 | -95.2 | +80.3 |
| *G. crassa* | HL | 7.92 | 18.93 | 5.78 | +139.0 | -69.5 |
| | IL | 7.92 | 17.45 | 4.59 | +120.3 | -73.7 |
| | LL | 7.92 | 8.32 | 5.41 | + 5.1 | -34.9 |
| *G.corticata* | HL | 80.05 | 55.68 | —— | -30.44 | -100% |
| | IL | 80.05 | 47.33 | —— | -40.9 | -100% |
| | LL | 80.05 | 2.96 | —— | -96.3 | -100% |

be higher under HL than under IL and LL treatments but the peak at 433 nm was more under IL on 6th day of treatment. The hump at 541 nm was prominent in all the light treatment conditions (Fig. 21). On the 12th day, samples under HL had prominent peaks. The peaks height at 495 nm showed marked variations under HL and IL but at 565 nm they were almost equal (Fig. 22). In *G. crassa* the peaks at 676, 495 and 433 nm were prominent under HL treatment but at 565 and 621 nm LL adapted plants are strong (Fig. 23). The peaks at 433 and 495 nm were reduced in their height under LL with a prominent shoulder at 450 nm and a sharp trough between 433 and 495 nm. The absorption spectrum below 420 nm decline sharply under LL treatment. The peak at 541 nm was not very prominent as seen in *G. edulis* (Fig. 24). In *G. corticata* the absorption spectra of 6 and 12 days treated samples did not show much variation. A shoulder around 433 nm was noticed in samples maintained under HL and IL. Low light treated plant did not show any prominent peaks on 6th day of treatment (Fig. 25). The trough between 495 and 433 nm for IL and HL treated plant was very sharp. A split peak was observed at 495 nm under HL, which becomes prominent after 12 days of treatment. In 12 days treated plant, the peak at 433 nm was shifted to 430 nm under IL. The shoulder at 450 nm becomes prominent (Fig. 26).

The fast fluorescence kinetic, showed much variation among species of *Gracilaria* subjected to different light intensity treatments for 6 and 12 days (Fig. 27). In control (untreated fresh) sample the variable fluorescence and quantum yield was maximum for *G. corticata* then in *G. crassa* and *G. edulis*. Upon exposure the values increased in all the treated samples of *G. edulis* and such increase was maximum only in sample kept under IL although there was variation on 6th and 12th day of treatment. On 12th day, the Fv and Fm/Fv values declined marginally under IL. In *G. crassa*, the variable fluorescene and quantum yield increased in IL but declined to a marked extent

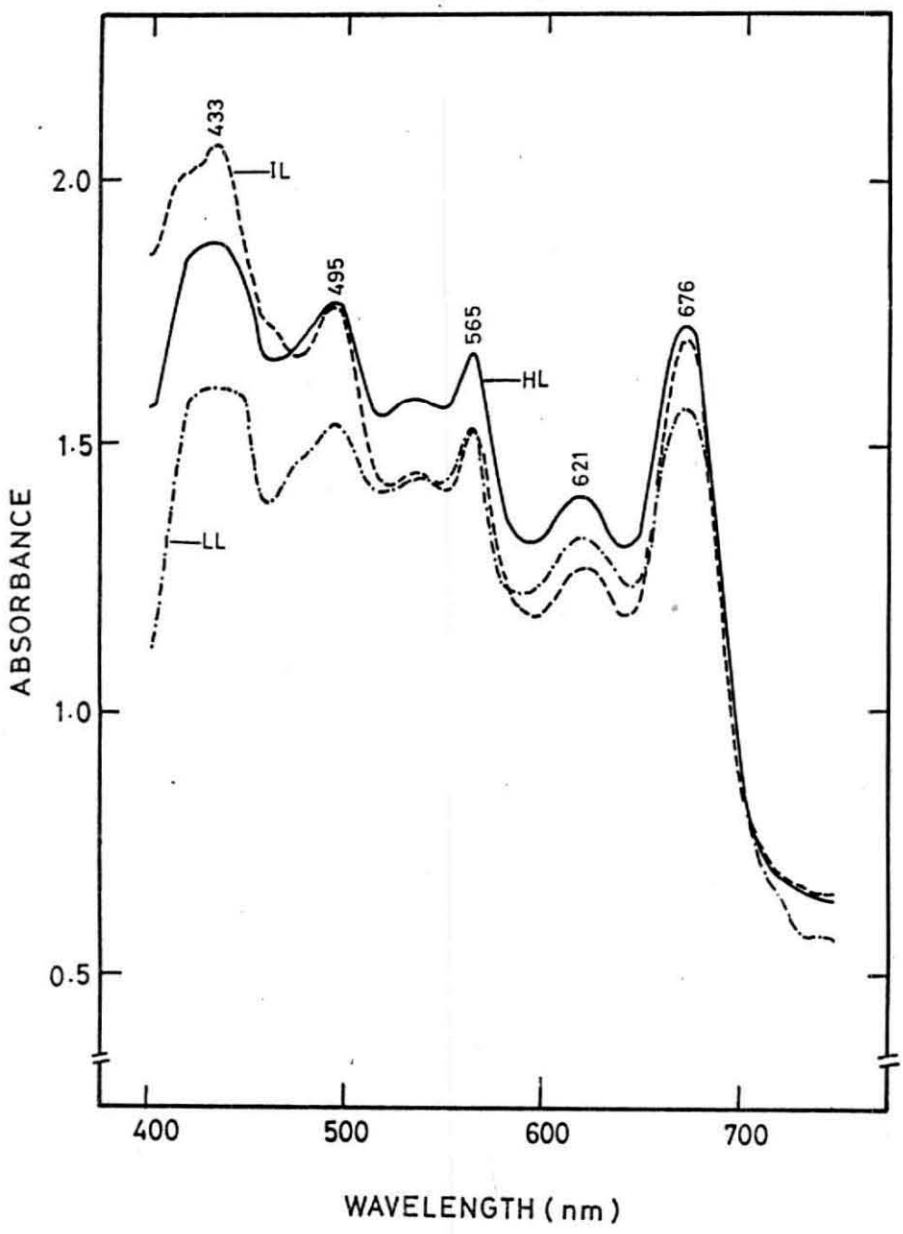


**Fig. 21**

Room temperature absorption spectra of the thallus of *Gracilaria edulis* after 6 days of treatment under different light intensities (LL, 0.5 W/m$^2$, IL, 2.0 W/m$^2$, HL, 3.0 W/m$^2$). For details of spectral measurements, see Materials and methods.

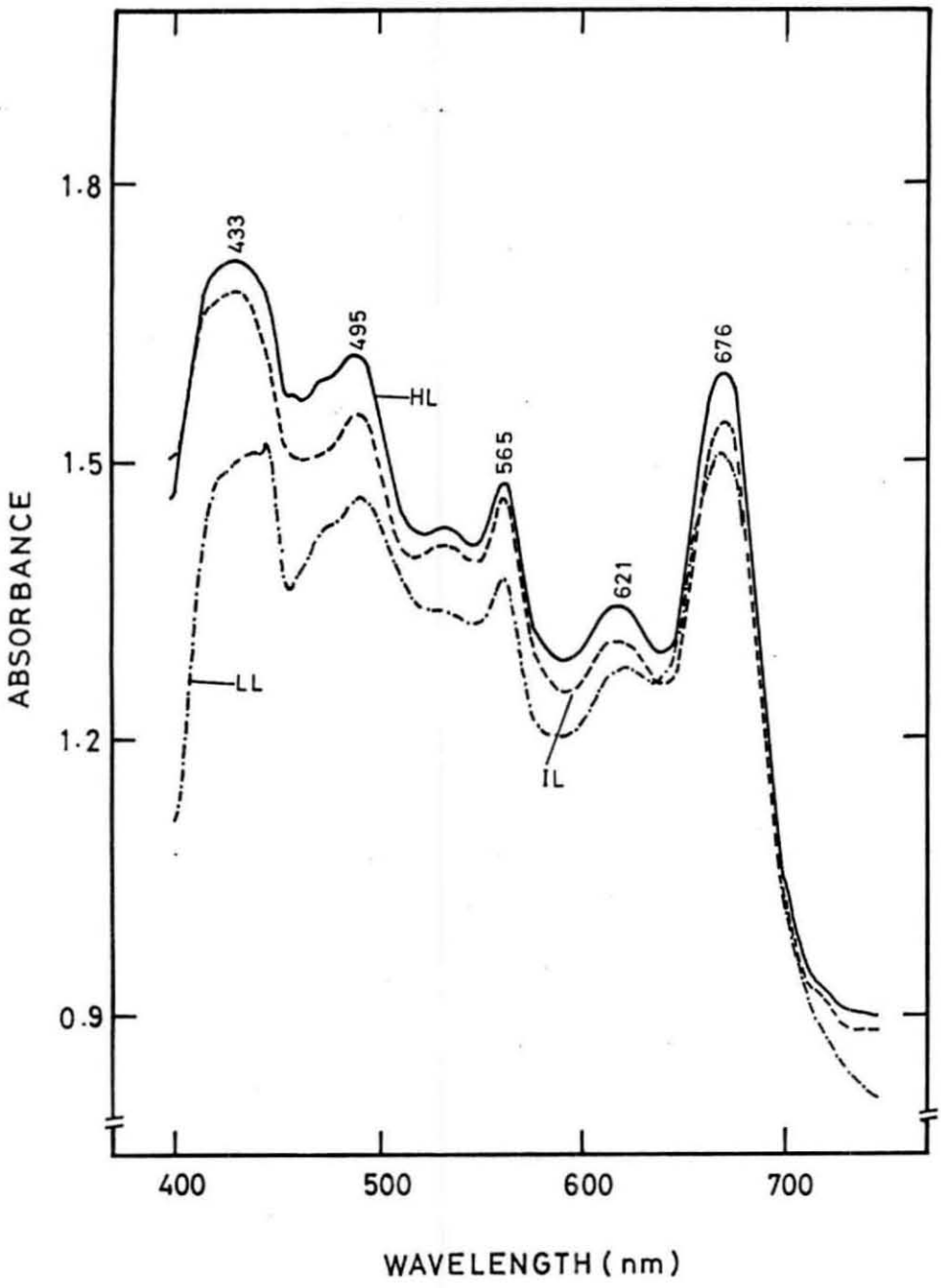


**Fig. 22**

Room temperature absorption spectra of the thallus of *Gracilaria edulis* after 12 days of treatment under different light intensities (LL, 0.5 W/m$^2$, IL, 2.0 W/m$^2$, HL, 3.0 W/m$^2$). For details of spectral measurements, see Materials and methods.

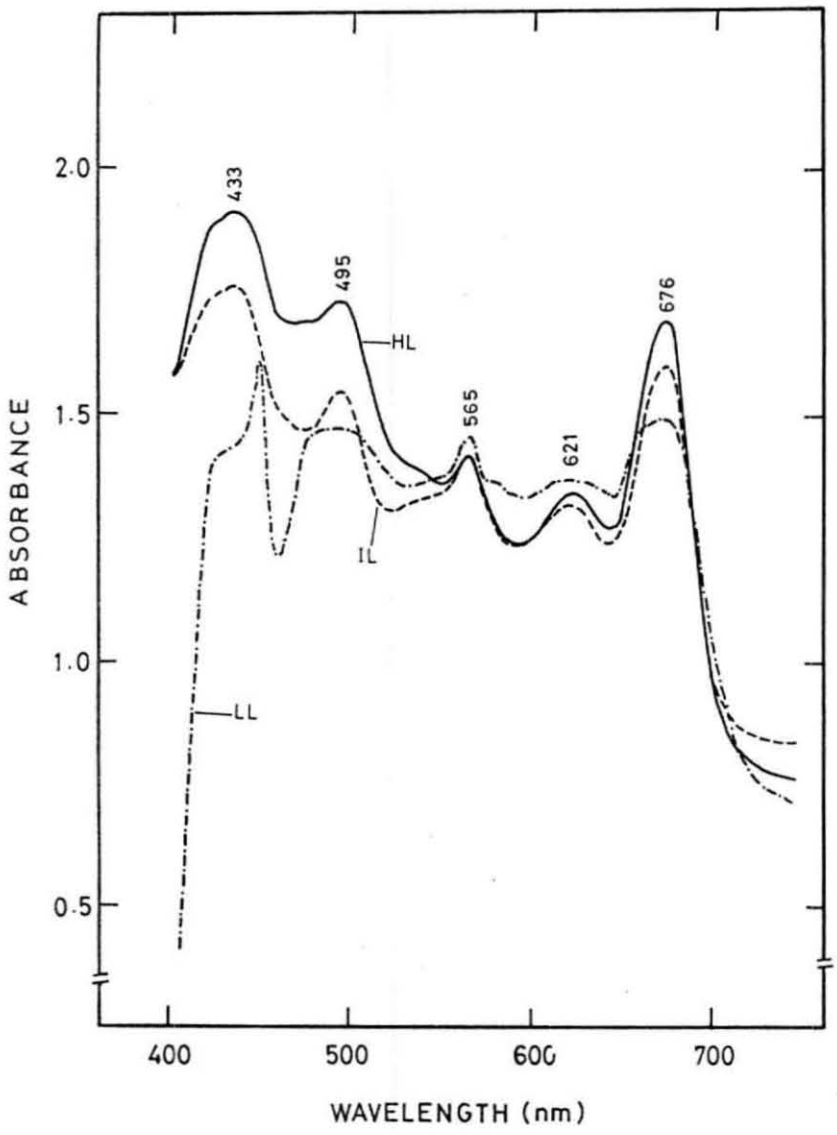


**Fig. 23**

Room temperature absorption spectra of the thallus of *Gracilaria crassa* after 6 days of treatment under different light intensities (LL, 0.5 W/m$^2$, IL, 2.0 W/m$^2$, HL, 3.0 W/m$^2$). For details of spectral measurements, see Materials and methods.

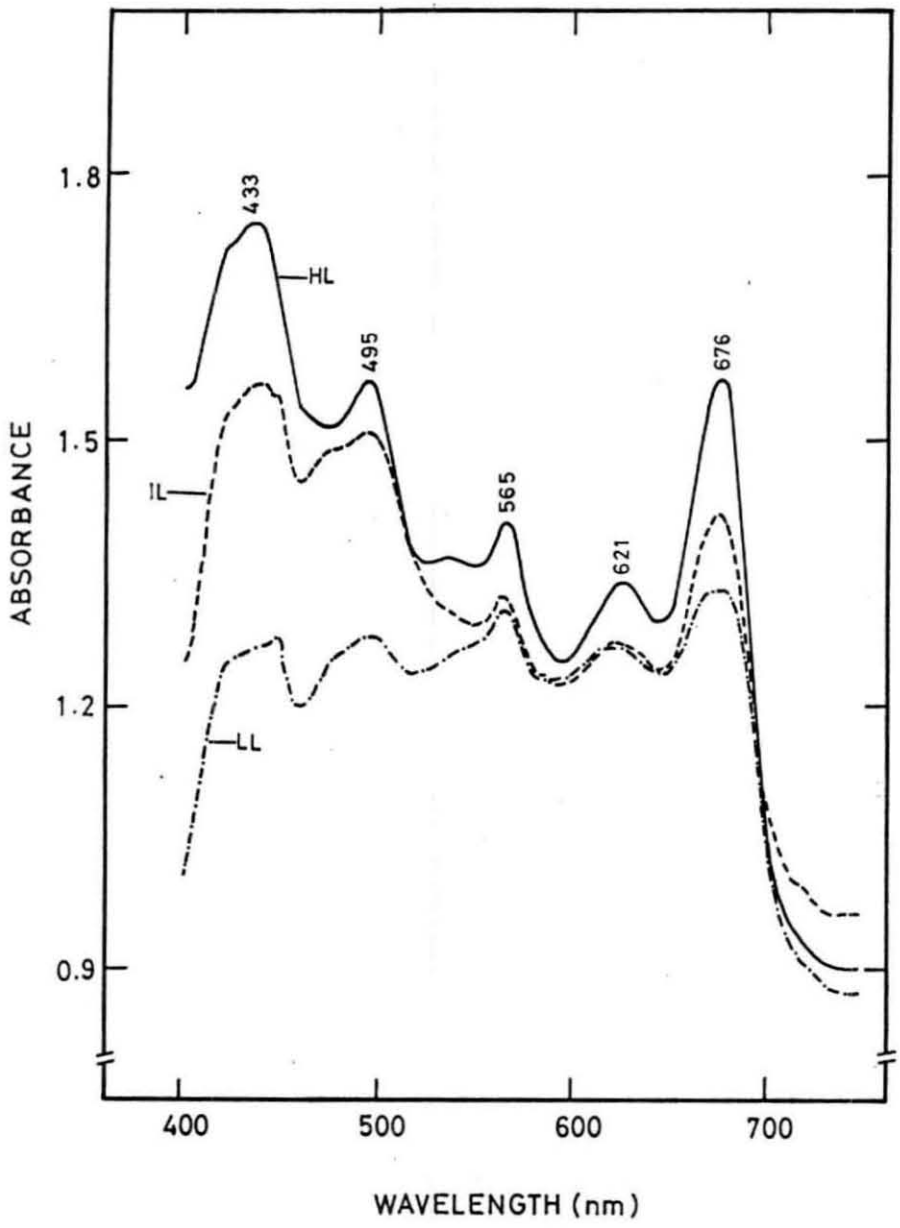


**Fig. 24**

Room temperature absorption spectra of the thallus of *Gracilaria crassa* after 12 days of treatment under different light intensities (LL, 0.5 W/m$^2$, IL, 2.0 W/m$^2$, HL, 3.0 W/m$^2$). For details of spectral measurements, see Materials and methods.

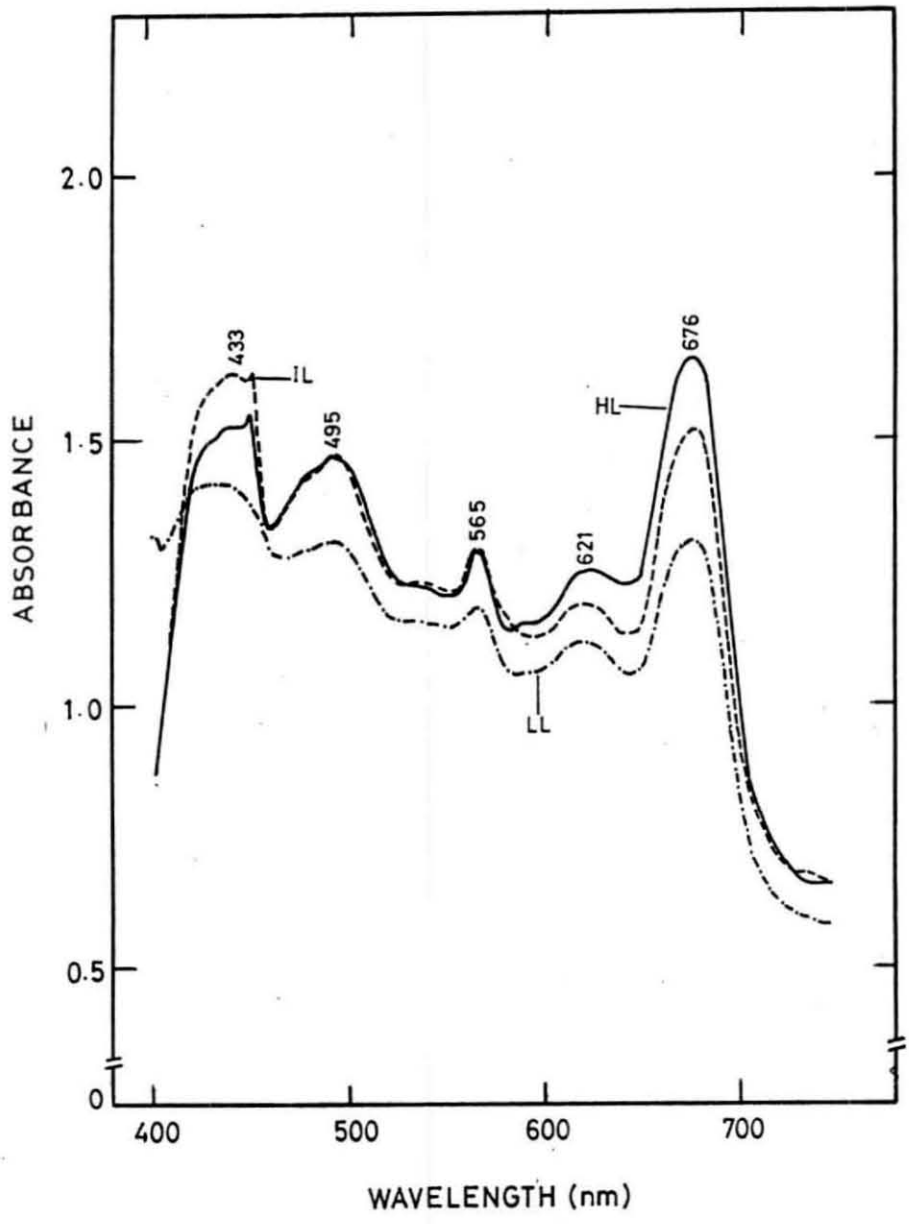


**Fig. 25**

Room temperature absorption spectra of the thallus of *Gracilaria corticata* after 6 days of treatment under different light intensities (LL, 0.5 W/m$^2$, IL, 2.0 W/m$^2$, HL, 3.0 W/m$^2$). For details of spectral measurements, see Materials and methods.

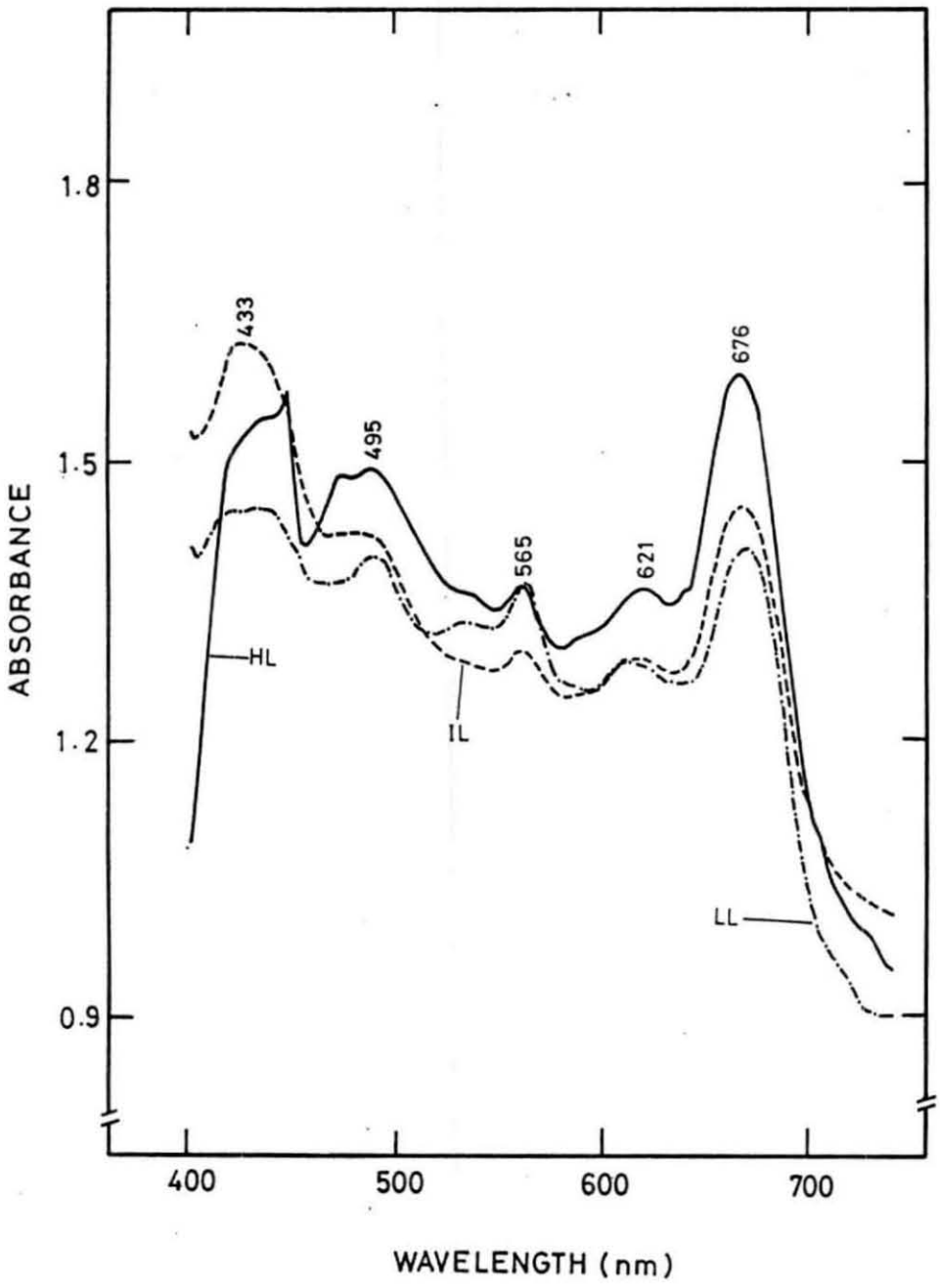


**Fig. 26**

Room temperature absorption spectra of the thallus of *Gracilaria corticata* after 12 days of treatment under different light intensities (LL, 0.5 W/m$^2$, IL, 2.0 W/m$^2$, HL, 3.0 W/m$^2$). For details of spectral measurements, see Materials and methods.

**Table 11**

Effect of light intensities on the fluorescence kinetics of Gracilaria spp.

| Species | Treatment | | Fast kinetics | | Slow kinetics | | |
|---|---|---|---|---|---|---|---|
| | | | Variable fluorescence | Quantum yield | Peak value | Terminal value | |
| | days | (ppt) | (Fv) | (Fv/Fm) | (P) | (T) | P-T |
| *G. edulis* | 0 | — | 2.1 | 0.21 | 6.2 | 4.7 | 1.5 |
| | 6 | 3.0 | 0.3 | 0.04 | 9.9 | 9.0 | 0.9 |
| | | 2.0 | 5.7 | 0.42 | 11.0 | 8.9 | 2.1 |
| | | 0.5 | 0.1 | 0.01 | 13.8 | 12.6 | 1.2 |
| | 12 | 3.0 | 0.9 | 0.10 | 7.9 | 7.2 | 0.7 |
| | | 2.0 | 4.7 | 0.37 | 9.0 | 7.2 | 1.8 |
| | | 0.5 | 1.3 | 0.14 | 5.8 | 4.8 | 1.0 |
| *G. crassa* | 0 | — | 4.3 | 0.35 | 11.9 | 9.7 | 2.2 |
| | 6 | 3.0 | 4.1 | 0.34 | 14.2 | 12.6 | 1.6 |
| | | 2.0 | 4.9 | 0.38 | 15.3 | 13.0 | 2.3 |
| | | 0.5 | 0.9 | 0.10 | 12.9 | 8.3 | 4.6 |
| | 12 | 3.0 | 3.1 | 0.28 | 11.3 | 9.3 | 2.0 |
| | | 2.0 | 3.5 | 0.30 | 9.6 | 7.6 | 2.0 |
| | | 0.5 | 3.9 | 0.33 | 14.9 | 11.9 | 3.0 |
| *G. corticata* | 0 | — | 8.2 | 0.51 | 13.4 | 10.4 | 3.0 |
| | 6 | 3.0 | 1.5 | 0.16 | 3.7 | 3.5 | 0.2 |
| | | 2.0 | 4.2 | 0.34 | 16.7 | 13.5 | 3.2 |
| | | 0.5 | 0.6 | 0.07 | 9.1 | 8.3 | 0.8 |
| | 12 | 3.0 | 2.8 | 0.26 | 10.2 | 9.1 | 1.1 |
| | | 2.0 | 3.0 | 0.27 | 10.2 | 7.9 | 2.3 |
| | | 0.5 | 2.2 | 0.22 | 16.0 | 13.7 | 2.3 |

under LL. In HL it did not show much variation. Further treatment resulted in decline of the above values under HL and IL but increased to a marked extent under LL. In *G. corticata* Fv and Fv/Fm values declined in all the treated samples on the 6th day. Maximum decline was noticed under IL. In LL and HL there was some recovery in fluorescence yield after 12 days of treatment but IL samples it declined further. As the initial rise was quite high, it maintained the higher fluorescence yield even after 12 days of treatment (Table 11).

Slow fluorescence kinetics exhibited variation in the peak and terminal values among the species and within different treatments. In the macroalgae, there was no definite P, M, S and T points as observed in higher plants (Fig. 28). In *G. edulis*, the peak and terminal values and their differences were much higher on 0 day and declined in all treated samples. Under IL the P-T values remain high on 6th and 12 th day of treatment. In *G. crassa*, the difference in P and T value increased in IL and LL on 6th day of treatment and declined marginally under HL. Further incuabtion reduced the value under IL and LL but recovered in HL. The values remain to be high in LL even after 12 days of treatment. In *G. corticata*, the P-T values declined under HL and LL but increased marginally in IL. Further incubation increased the fluorescence response in HL and IL which is highly pronounced under LL (Table 11).

**Effect of light quality**

*G. edulis*, *G. corticata* and *G. crassa* were exposed to different broad band lights (blue, green, red and white) in a growth chamber, and the changes in their pigment composition and photosynthetic activity were followed.

The Chl content of *G. edulis* increased in all the treatment after 6 days of exposure. Maximal increase was noticed under green light (GL) compared to white (WL) and red light (RL). After 12 days of treatment, there was

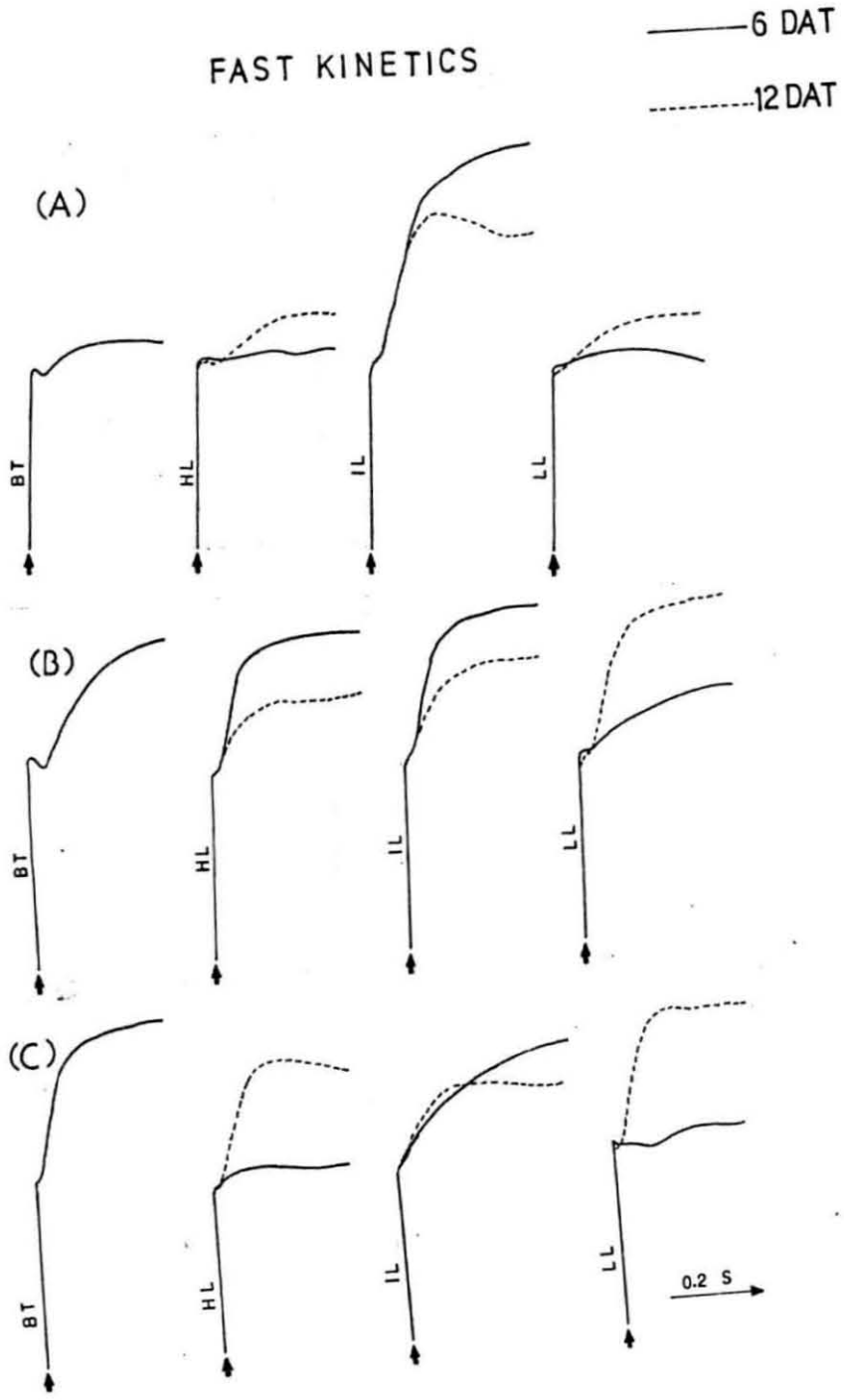


**Fig. 27**

Typical fast fluorescence kinetics of *Gracilaria edulis* (A), *G. crassa* (B) and *G. corticata* (C) subjected to treatments under different light intensities for 6 and 12 days (LL, 0.5 W/m², IL, 2.0 W/m², HL, 3.0 W/m²). For details of fluorescence measurements, see Materials and methods.

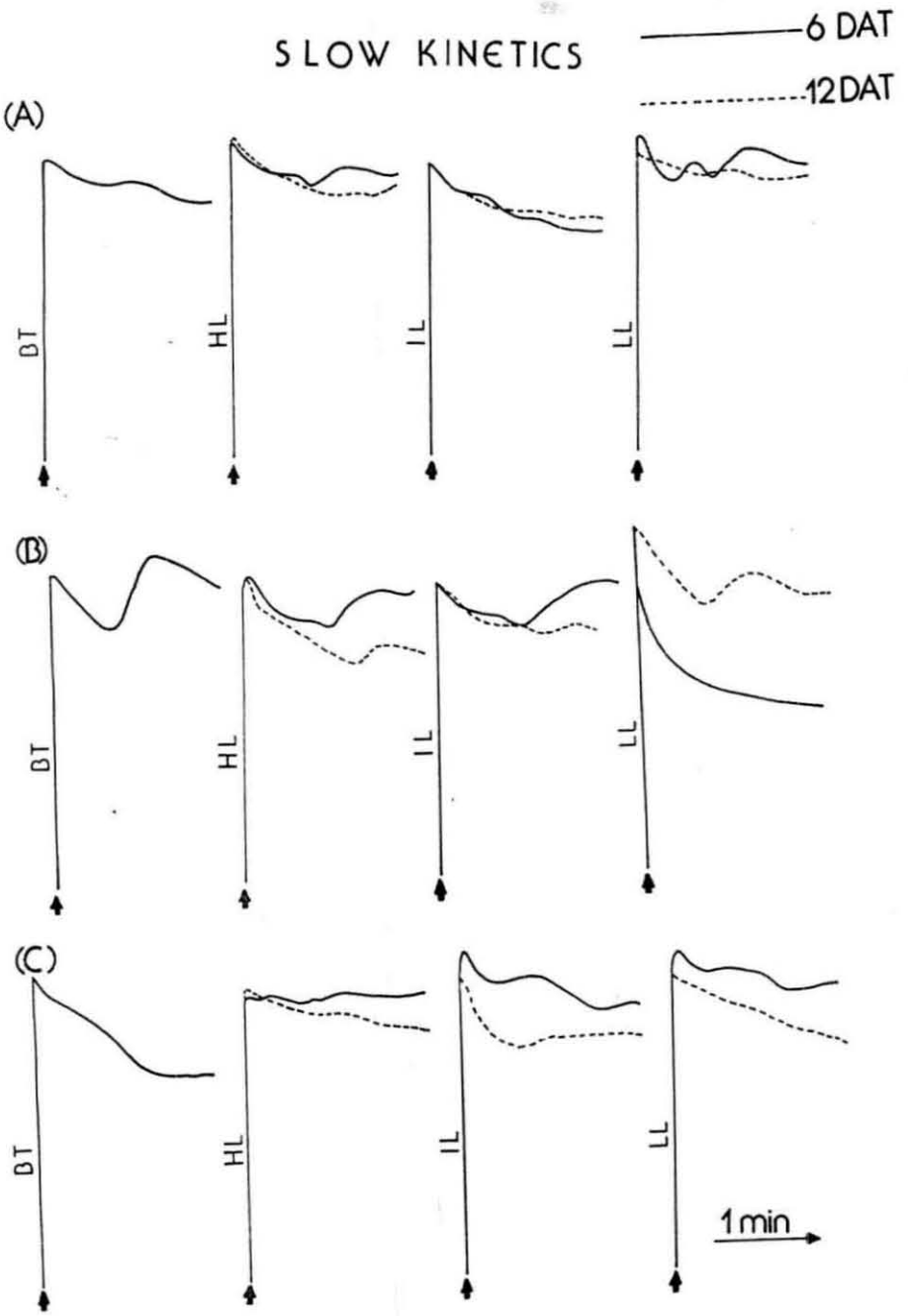


**Fig. 28**

Typical slow fluorescence kinetics of *Gracilaria edulis* (A), *G. crassa* (B) and *G. corticata* (C) subjected to treatments under different light intensities for 6 and 12 days (LL, 0.5 W/m$^2$, IL, 2.0 W/m$^2$, HL, 3.0 W/m$^2$). For details of fluorescence measurements, see Materials and methods.

a marginal decline in Chl content in WL but significantly under GL. Whereas under BL and RL there was a gradual increase in Chl content. In *G. corticata* Chl content on 6th day had increased to a greater extent compared to *G. edulis*. Maximum increase was noticed under GL followed by WL, RL and BL. Similar to *G. edulis*, further increase of Chl content was noticed only under BL and RL on 12 days of treatment. Both WL and GL had caused a decrease. In *G. crassa*, the Chl content declined in all the treatments except a marginal increase of 7.4% under RL. Maximum decline was noticed under WL (58.5%) (Table 12).

PE content of *G. edulis* registered an increase on the 6 day of treatment in all the samples. It varied from 21% in WL to 61.5% in GL. On the 12th day of treatment, the PE content declined in GL but increased in all other light treatments. RL and BL had increased the PE content by 14.3% and 34.2%, respectively. However, GL exhibited a 20.5% decline over the control (WL) on the 12th day of treatment.

In *G. corticata* the PE content showed an increase in GL, followed by RL and BL but it declined in WL on 6th day of treatment. Further, on the 12th day of treatment, PE content declined under WL and BL. Under RL, there was only a marginal decline (2.6%) of PE pigment. Whereas in GL it continued to increase further with reference to control, all the treated samples showed more PE content on the 6th as well as on the 12th day of treatment and maximum was noticed under GL (Table 13).

In *G. crassa*, inititally the PE content increased marginally in all the treatment condition execpt under RL where it showed a decline (by 17%). WL had produced an increase of 32.5% on 6th day of treatment. Further on 12th day, the PE content increased in all the treated samples except under WL where a decline by 32.1% was observed. With reference to control, the PE content was found to be less by 23% in GL, 25.2% in BL and 37.4%

**Table 12**

Effect of light qualities on chlorophyll content of *Gracilaria* spp.

| Species | Treatment | Chlorophyll content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | WL | 0.0531 | 0.2036 | 0.1998 | +283.4 | - 1.87 |
| | GL | 0.0531 | 0.2132 | 0.1814 | +301.5 | -14.92 |
| | BL | 0.0531 | 0.1480 | 0.1786 | +178.7 | +25.1 |
| | RL | 0.0531 | 0.1493 | 0.1868 | +181.4 | +20.8 |
| *G. crassa* | WL | 0.0646 | 0.1084 | 0.0450 | + 67.8 | -58.5 |
| | GL | 0.0646 | 0.0960 | 0.0810 | + 48.6 | -15.5 |
| | BL | 0.0646 | 0.1162 | 0.1121 | + 79.9 | - 3.5 |
| | RL | 0.0646 | 0.0863 | 0.0927 | + 33.6 | + 7.4 |
| *G.corticata* | WL | 0.0680 | 0.4136 | 0.0941 | +508.2 | -77.2 |
| | GL | 0.0680 | 0.4828 | 0.1736 | +610.0 | -64.0 |
| | BL | 0.0680 | 0.1348 | 0.2188 | + 98.2 | +62.3 |
| | RL | 0.0680 | 0.2048 | 0.2232 | +210.2 | + 8.98 |

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**Table 13**

Effect of light qualities on phycoerythrin content of *Gracilaria* spp.

| Species | Treatment | Phycoerythrin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | WL | 0.3959 | 0.4828 | 0.5832 | + 21.0 | +20.8 |
| | GL | 0.3959 | 0.6441 | 0.4638 | + 61.5 | -32.2 |
| | BL | 0.3959 | 0.5594 | 0.7829 | + 40.2 | +39.9 |
| | RL | 0.3959 | 0.6014 | 0.6630 | + 50.8 | +10.8 |
| *G. crassa* | WL | 0.4502 | 0.5965 | 0.4049 | + 32.5 | -32.1 |
| | GL | 0.4502 | 0.4588 | 0.5427 | + 1.9 | +18.3 |
| | BL | 0.4502 | 0.4460 | 0.4628 | - 0.9 | + 4.4 |
| | RL | 0.4502 | 0.3736 | 0.4869 | - 17.0 | +30.3 |
| *G.corticata* | WL | 0.5355 | 0.3425 | 0.2782 | - 36.0 | -18.8 |
| | GL | 0.5355 | 0.7128 | 0.8366 | + 33.1 | +17.4 |
| | BL | 0.5355 | 0.5374 | 0.5234 | + 0.4 | - 2.6 |
| | RL | 0.5355 | 0.6156 | 0.4947 | + 14.9 | -19.6 |

in RL on 6th day but on 12th day all the treated samples had more PE than the control. Maximum increase was noticed under GL (34.0%) followed by RL (20.3%) and BL 15.0%.

Phycocyanin content was also found to be more on the 6th day of treatment in *G. edulis* and *G. corticata* except for a decline of 25.6% under WL in *G. corticata*. With reference to control, the PC content was found to be more in treated samples. Maximum increase was observed under GL followed by BL and RL. On the 12th day, the PC content declined in all the treated samples except under RL which exhibited a further increase. WL caused a marginal decline of 2.8% compared to 25% and 12.7% in GL and BL light, respectively. On 12th day, *G. corticata* too registered a decline of PC content in all treatment conditions except for a marginal increase under BL. Maximum reduction was noticed under RL compared to WL and GL. Both *G. edulis* and *G. corticata* showed decline of PC content under all treatment conditions. Maximum decline was noticed under BL.

In *G. crassa*, the PC content was found to be more under WL than under GL but BL and RL decreased in level by 8.2% and 9.6%, respectively. On 12th day, this trend was reversed reducing PC content in WL and GL but increasing under BL and RL. With reference to control, the treated sample exhibited more PC content. BL registered the maximum increase compared to other treated samples (Table 14).

APC content of *G. edulis* gradually increased in all the treated samples on till 12 days except for a decline of 20.6% under GL on 12th day with reference to the level on 6th day. However, in general, the APC content increased in all the treated sample with reference to 0 day. In *G. corticata* the APC content was found to be maximum in GL on 6th and 12th day of treatment. APC content found to increase in all the treated sample but declined in control on 6th day of treatment. The APC content continued to increase

**Table 14**

Effect of light qualities on phycocyanin content of *Gracilaria* spp.

| Species | Treatment | Phycocyanin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | WL | 0.1984 | 0.2914 | 0.2834 | + 46.9 | - 2.8 |
| | GL | 0.1984 | 0.4888 | 0.3668 | +146.4 | -25.0 |
| | BL | 0.1984 | 0.4536 | 0.3959 | +128.6 | -12.7 |
| | RL | 0.1984 | 0.3129 | 0.3716 | + 57.7 | +18.8 |
| *G. crassa* | WL | 0.2523 | 0.3796 | 0.2297 | + 50.5 | -39.5 |
| | GL | 0.2523 | 0.2807 | 0.2664 | + 11.3 | - 5.1 |
| | BL | 0.2523 | 0.2315 | 0.2893 | - 8.2 | +24.9 |
| | RL | 0.2523 | 0.2280 | 0.2725 | - 9.6 | +19.5 |
| *G.corticata* | WL | 0.2036 | 0.1515 | 0.0980 | - 25.6 | -35.3 |
| | GL | 0.2036 | 0.3525 | 0.2613 | + 73.1 | -25.9 |
| | BL | 0.2036 | 0.2620 | 0.2800 | + 28.7 | + 6.9 |
| | RL | 0.2036 | 0.3158 | 0.1944 | + 55.1 | -38.4 |

in GL till 12th day of treatment whereas it declined in BL and RL. Recovery in APC content was noticed in control.

In *G. crassa*, the APC content was maximum in WL on 6th day but on 12th day, GL treated samples showed the maximum APC content compared to others. Initially there was an increase of APC content in all the treated samples compared to 0 day treatment. Such increase was maximum under WL (49.9%) compared to marginal increase in other treatments. Further treatment, decline the APC content only under WL by 30.6% but continued to increase in other treatments (Table 15).

The photosynthetic activity was found to be maximum in *G. corticata* among the three species. Upon exposure to different light conditions, *G. edulis* exhibited an increase in activity in WL, GL, and BL but declined by 19.9% in RL on the 6th day. Maximum increase was observed under WL. On 12th day, the activity was found to be reduced in all the samples except those under BL. In *G. corticata* maximum decline of photosynthetic activity was noticed and most of the treated samples showed oxygen uptake on 12th day except in RL. In *G. crassa*, the photosynthetic rate was increased in WL and GL on 6th day but declined thereafter. Under BL the activity continued to decline gradually till 12th day (Table 16).

The absorption spectrum of fresh incubated thalli of *G. edulis*, *G. crassa* and *G. corticata* showed many peaks as described earlier (Fig. 20). The peak at 541 nm was more prominent for *G. edulis* compared to other two species. In *G. edulis* under WL, the peak at 433 was highly elevated forming more number of peaks from 433 to 495 nm. In BL, the trough near 433 nm was most prominent on 6th day of treatment. GL showed more peaks between 433-495 nm as was observed under WL on the 6th day (Fig. 29). The peak at 495 nm was shifted to 500 nm in WL after 12 days of treatment. The

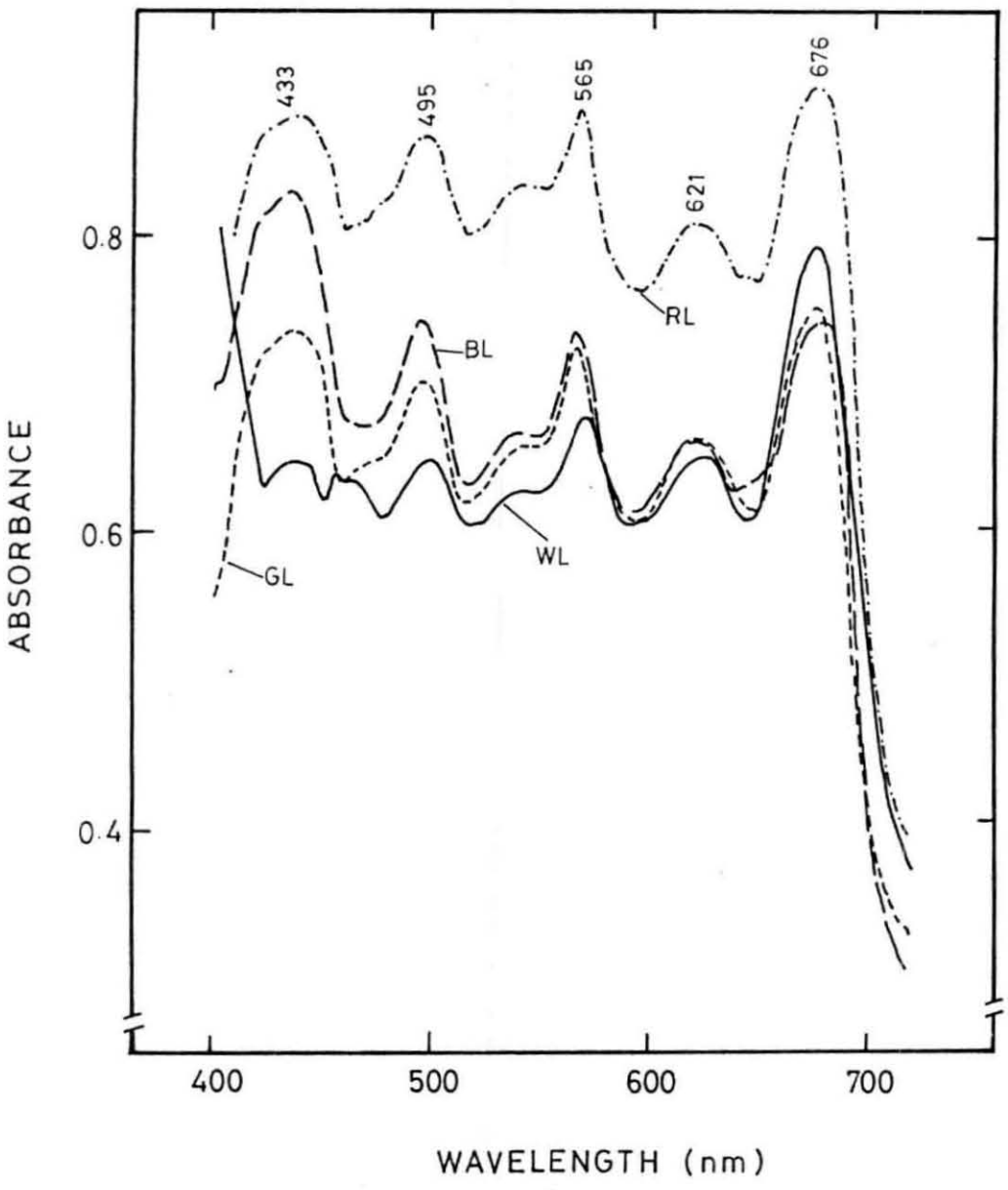


**Fig. 29**

Room temperature absorption spectra of the thallus of *Gracilaria edulis* after 6 days of treatment under different broad band monochromatic and white lights. RL red light; BL, blue light; GL, green light; WL, white light. For the treatment condition and spectral measurements, see Materials and methods.

**Table 15**

Effect of light qualities on allophycocyanin content of *Gracilaria* spp.

| Species | Treatment | Allophycocyanin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | WL | 0.1686 | 0.2974 | 0.3582 | + 76.4 | +20.4 |
| | GL | 0.1686 | 0.3612 | 0.2869 | +114.2 | -20.6 |
| | BL | 0.1686 | 0.2852 | 0.4739 | + 69.2 | +66.2 |
| | RL | 0.1686 | 0.3058 | 0.4613 | + 81.4 | +50.9 |
| *G. crassa* | WL | 0.2628 | 0.3940 | 0.2736 | + 49.9 | -30.6 |
| | GL | 0.2628 | 0.2780 | 0.3513 | + 5.8 | +26.4 |
| | BL | 0.2628 | 0.2793 | 0.2953 | + 6.3 | + 5.7 |
| | RL | 0.2628 | 0.2775 | 0.2800 | + 5.6 | + 0.9 |
| *G.corticata* | WL | 0.1866 | 0.1377 | 0.1765 | - 26.2 | +26.7 |
| | GL | 0.1866 | 0.3598 | 0.5448 | + 92.8 | +51.4 |
| | BL | 0.1866 | 0.2751 | 0.2642 | + 47.4 | - 3.9 |
| | RL | 0.1866 | 0.2935 | 0.1886 | + 57.3 | -35.7 |

**Table 16**

Effect of light qualities on photosynthetic activity of *Gracilaria* spp.

| Species | Treatment | Photosynthetic activity (µg/g Fw/ h) | | | % of change | |
|---|---|---|---|---|---|---|
| | | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | WL | 5.48 | 17.16 | 4.65 | +213.1 | -72.9 |
| | GL | 5.48 | 8.97 | 1.80 | + 63.7 | -79.9 |
| | BL | 5.48 | 12.32 | 1.56 | +124.8 | -87.3 |
| | RL | 5.48 | 4.39 | 7.01 | - 19.9 | +59.9 |
| *G. crassa* | WL | 5.24 | 13.32 | 3.72 | +154.2 | -72.1 |
| | GL | 5.24 | 6.27 | 3.06 | + 19.7 | -51.2 |
| | BL | 5.24 | 4.29 | 3.75 | - 18.1 | -12.6 |
| | RL | 5.24 | 5.25 | 2.16 | + 0.2 | -58.9 |
| *G.corticata* | WL | 6.69 | 6.16 | —— | - 7.9 | -100% |
| | GL | 6.69 | 5.47 | —— | -18.2 | -100% |
| | BL | 6.69 | 7.59 | —— | +13.5 | -100% |
| | RL | 6.69 | 3.80 | 4.86 | -43.2 | +27.9 |

peak at 541 nm was most prominent under RL than in other treated samples. The peak at 565 nm was shifted to 561 nm in WL, and 676 to 678 nm in RL (Fig. 30).

*G. crassa* showed differences in the spectra after treatment under different lights. On 6th day, shoulders were noticed at 450 nm in RL which were more prominent under WL. The trough between 433 and 495 nm were found to be flat under GL and BL (Fig. 31). On 12th day under RL, the spectrum declined sharply below 420 nm with a prominent shoulder at 450 nm. The peak at 495 nm was negligible with a flat peak at 621 nm under WL, the trough between 433 and 495 nm was conspicuously flat. The elevation at 541 nm were prominent under WL and RL but absent in GL and BL (Fig. 32).

In *G. corticata*, the spectrum was smooth with a prominent elevation at 541 nm in untreated samples. Upon treatment for 6 days, RL dominated the peaks at 676, 495 and 433 nm (Fig. 33). The humps at 541 nm was not as prominent as those in 0 day sample, and absent under WL. BL treatment produced number of peaks and crests between 433 and 495 nm. A prominent shoulder was noticed at 450 nm in WL. RL treatment shifted the 676 peak to 672 nm. BL treatment caused a sharp decline of absorbance at 400 nm. Maximum decline was noticed under WL. On 12th day, the spectrum remained almost similar except for the presence of flat trough between 433 and 495 nm in RL and WL. The peak at 676 nm was shifted to 670 nm in WL and GL. RL exhibited the shift of peak from 621 to 615 nm (Fig. 34).

**Effect of salinity**

To study the effect of salinity on pigments and physiological activities of *Gracilaria* plants were suspended in saline solution of 15, 25, 35 and 45 ppt and incubated for a maximum period of 12 days under controlled condition.

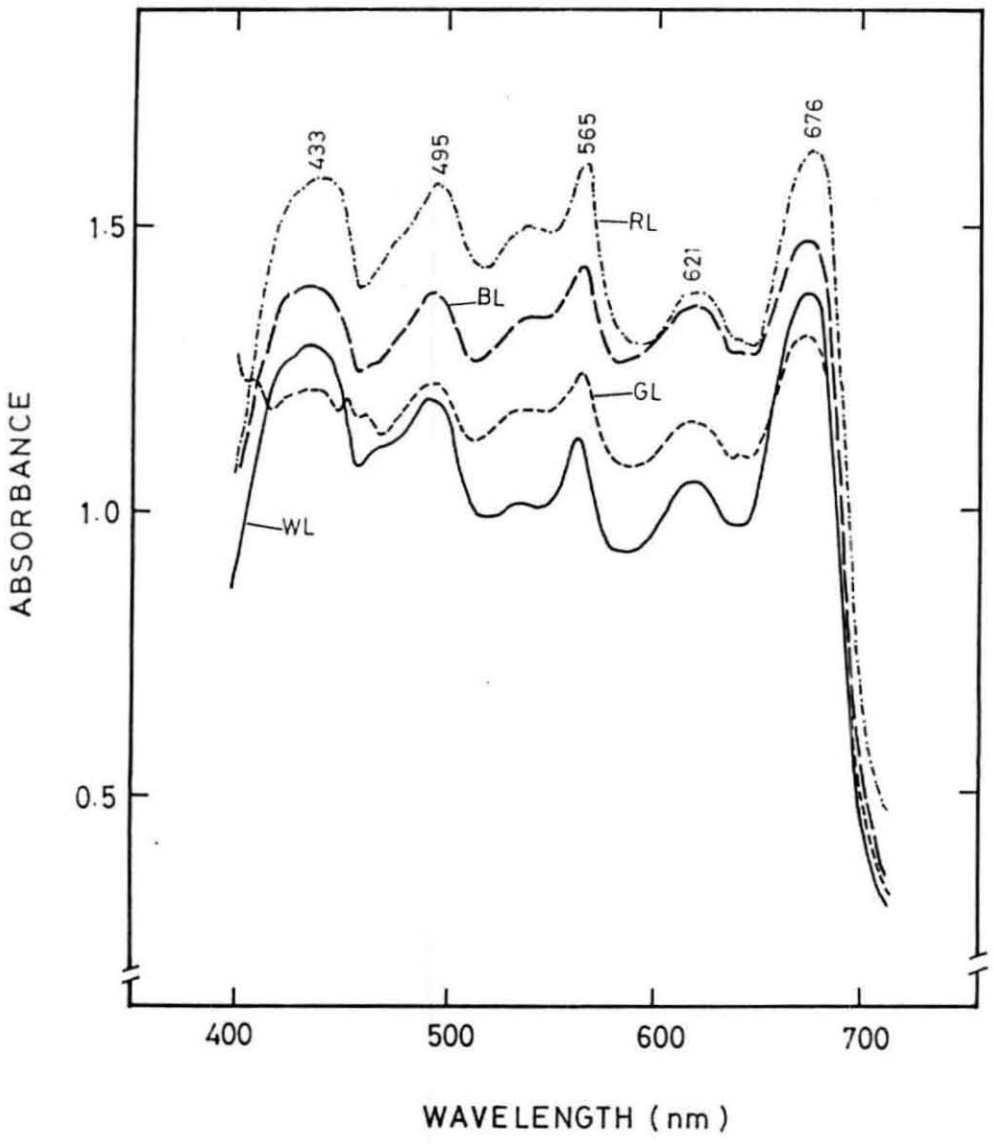


**Fig. 30**

Room temperature absorption spectra of the thallus of *Gracilaria edulis* after 12 days of treatment under different broad band monochromatic and white lights. RL red light; BL, blue light; GL, green light; WL, white light. Other details as in Fig. 33.

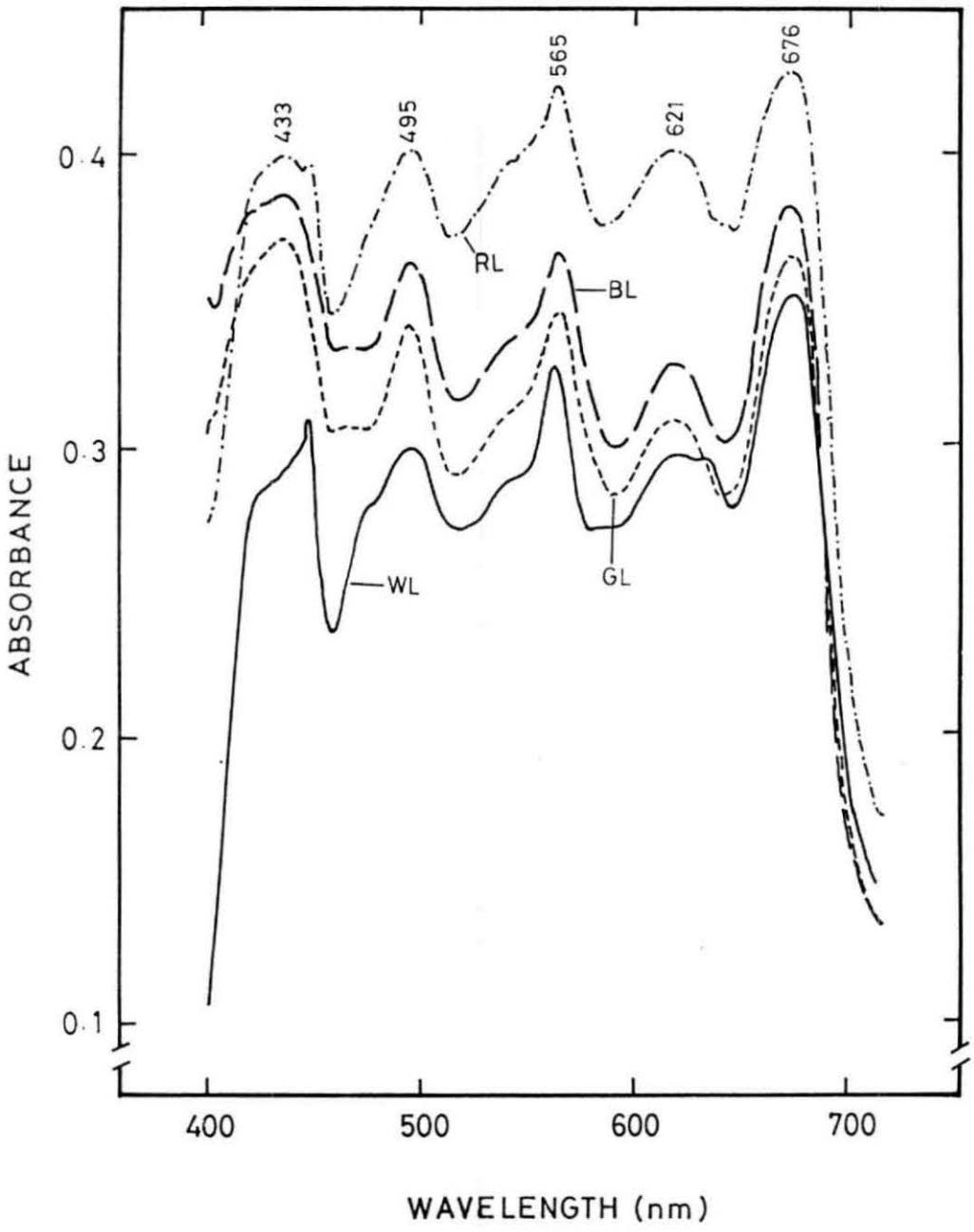


**Fig. 31**

Room temperature absorption spectra of the thallus of *Gracilaria crassa* after 6 days of treatment under different broad band monochromatic and white lights. RL red light; BL, blue light; GL, green light; WL, white light. Other details as in Fig. 33.

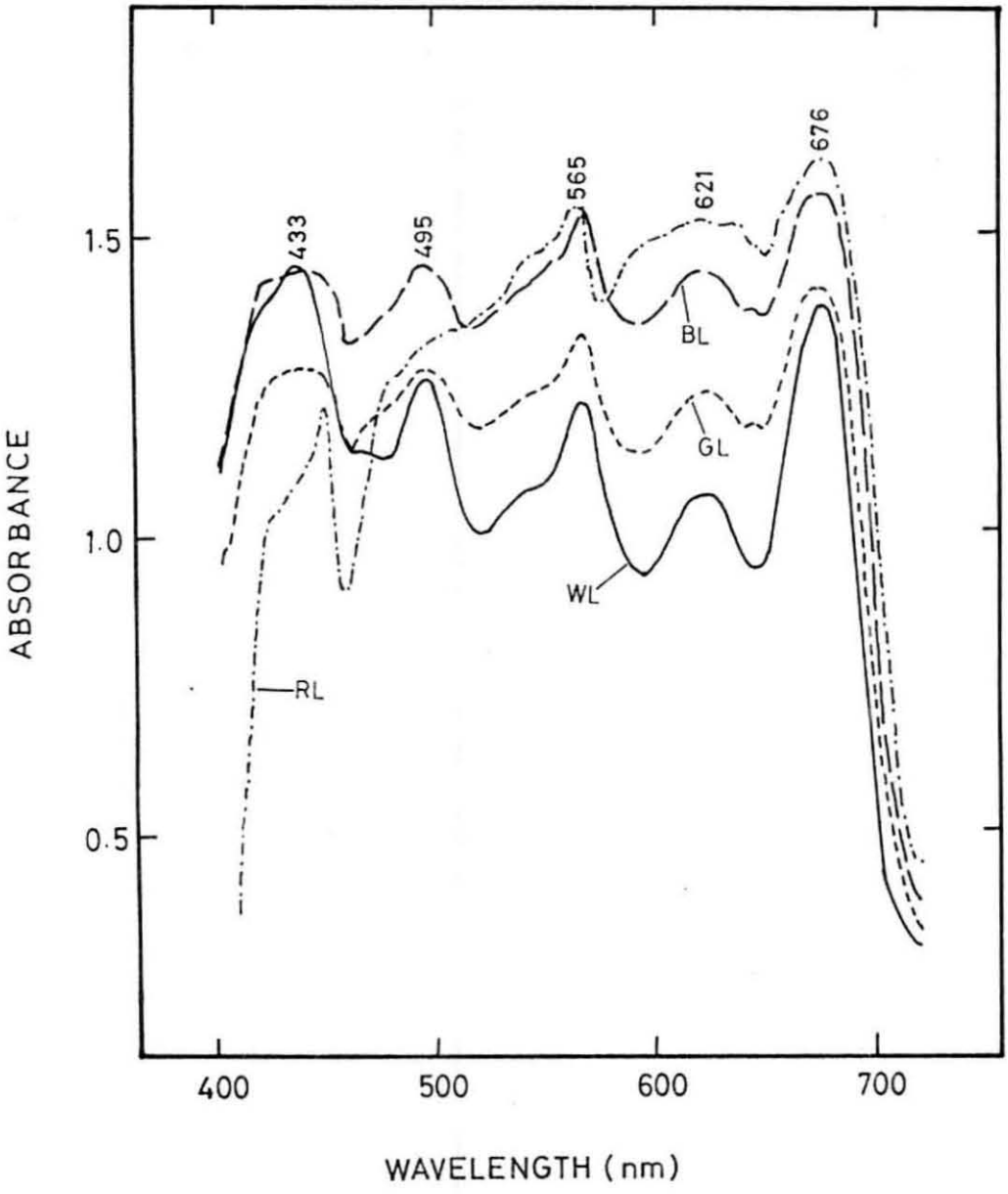


**Fig. 32**

Room temperature absorption spectra of the thallus of *Gracilaria crassa* after 12 days of treatment under different broad band monochromatic and white lights. RL red light; BL, blue light; GL, green light; WL, white light. Other details as in Fig. 33.

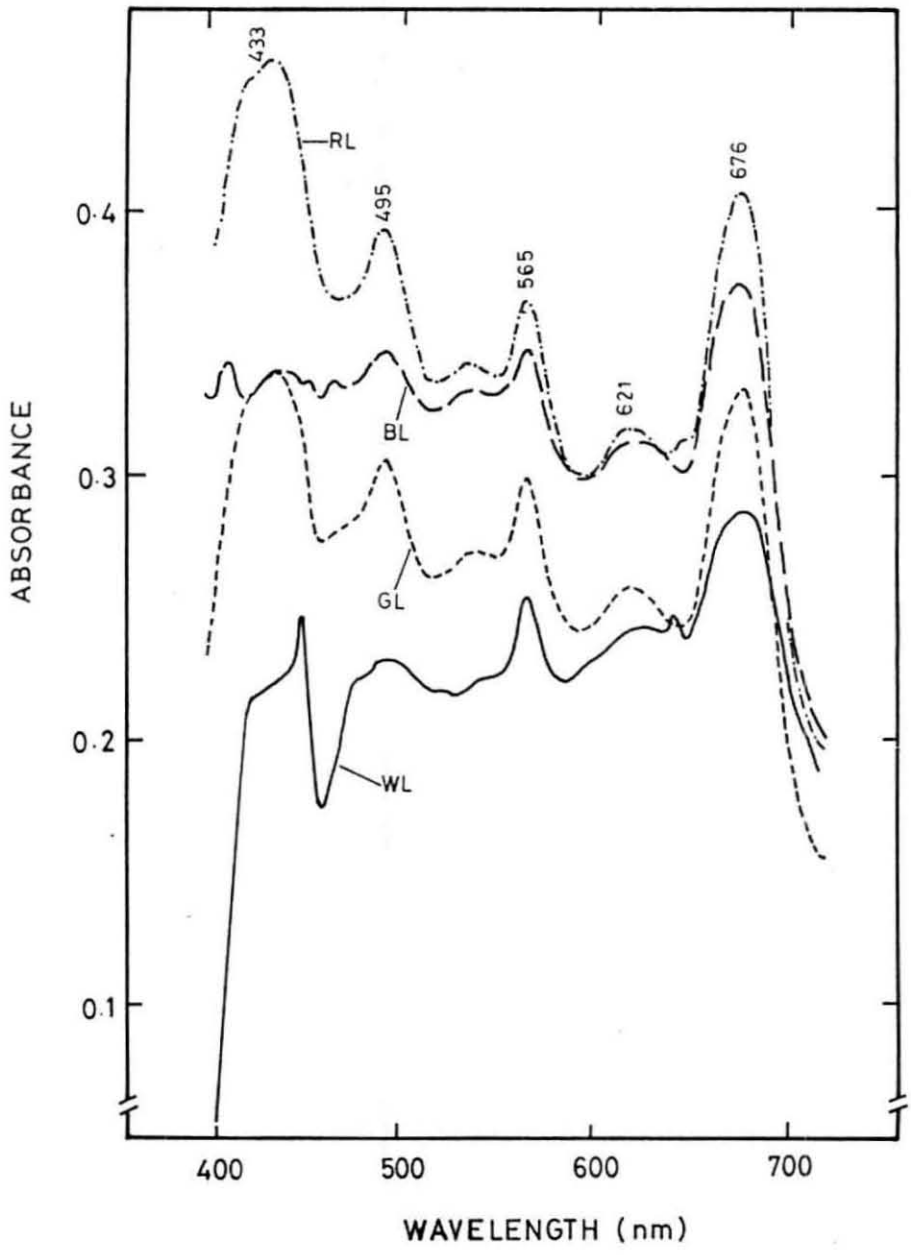


**Fig. 33**

Room temperature absorption spectra of the thallus of *Gracilaria corticata* after 6 days of treatment under different broad band monochromatic and white lights. RL red light; BL, blue light; GL, green light; WL, white light. Other details as in Fig. 33.

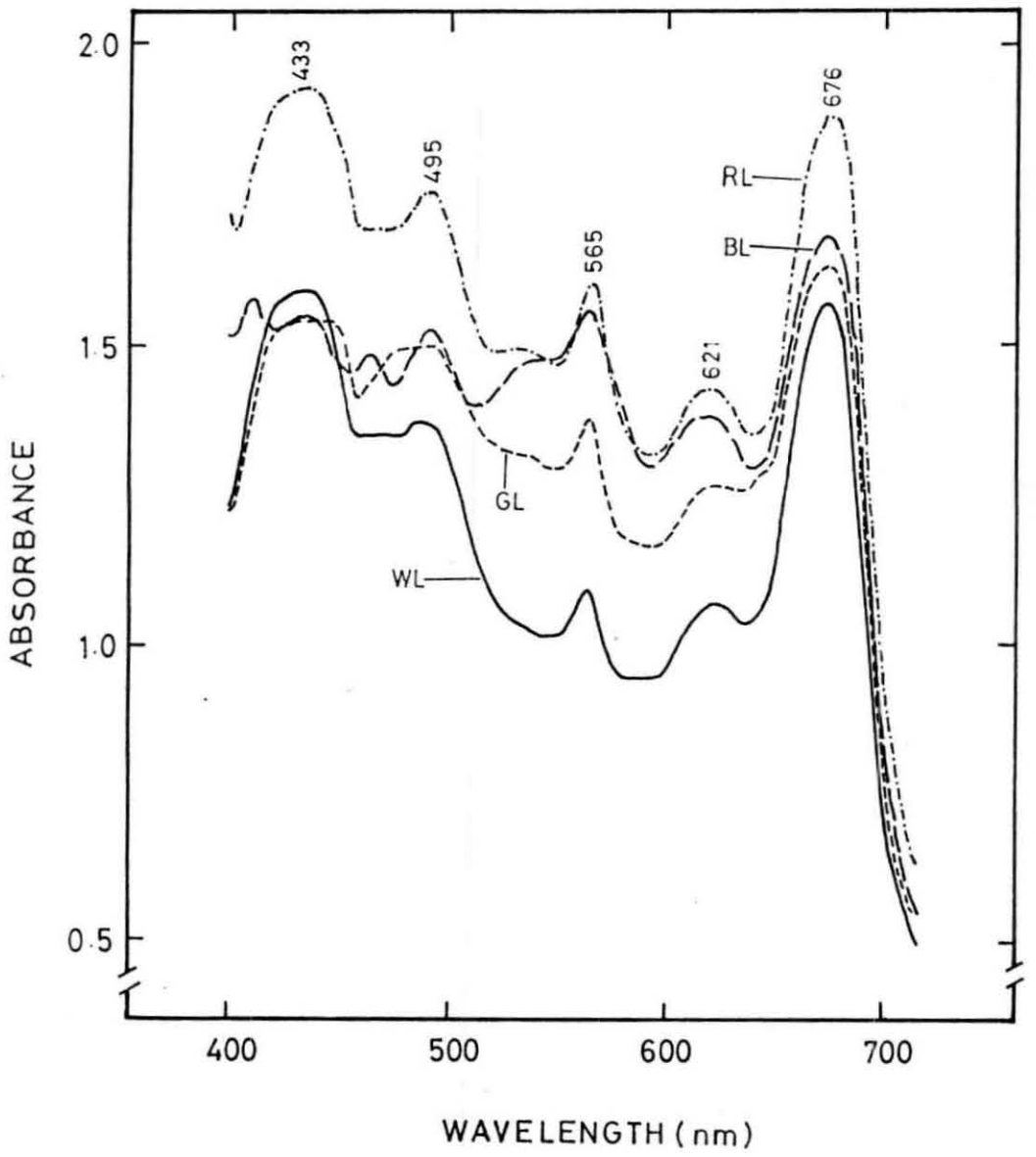


**Fig. 34**

Room temperature absorption spectra of the thallus of *Gracilaria corticata* after 12 days of treatment under different broad band monochromatic and white lights. RL red light; BL, blue light; GL, green light; WL, white light. Other details as in Fig. 33.

During this period the temperature and light intensity were maintained under optimal levels. Changes in the level of various pigments in *G. edulis* and *G. crassa* incubated under different saline solutions are presented in Table . After 6 days of treatment, *G edulis* showed a decline in Chl content at 15, 25 and 45 ppt levels. Such decrease was in the range of 25-50% from the initial level. However, sample kept at 35 ppt showed an increase of 60% over the initial level. In *G. crassa* the Chl content declined by 21.6% and 27.9% in hypersaline (45 ppt) and hyposaline (15 ppt) conditions, respectively. Whereas, it increased in 25 and 35 ppt. On 12th day in *G. edulis*, the Chl content declined in 15 and 35 ppt but increased at 25 and 45 ppt. *G. crassa* exhibited decline of Chl content after 12 days in all the treated samples (Table 17).

In *G. edulis* at 25 ppt, the level of all accessory pigments showed an increase. Similar trend was also seen in 45 ppt. However, at 15 and 35 ppt, the accessory pigments showed varied response. At 35 ppt both PC and APC contents increased but in 15 ppt only APC showed an increase by 9.9%. PE registered a decline in both the treatment (Table 18). In *G. crassa* the accessory pigments were found to increase in all the treated samples except for a decline in PC content at 15 and 45 ppt by 19.1% and 7.0%, respectively (Table 19). After 12 days of treatment the accessory pigments in all treatment conditioned have declined in both *G. edulis* and *G. crassa*. Such decline in *G. crassa* was more pronounced than in *G. edulis* (Table 20).

The photosynthetic activity was found to decrease in both *G. crassa* and *G. edulis* after 6 and 12 days of treatments and such decrease was more at low salt concentrations than under normal or high salt concentrations. In all the treatments, the activity declined gradually during the first 6 days of incubation and further till 12th of treatment. In 35 ppt the declined was marginal, being

**Table 17**

Effect of salinity on chlorophyll content of Gracilaria spp.

| Species | Treatment | Chlorophyll content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | ppt | 0 day | 6 days | 2 days | 0-6 days | 6-12 days |
| *G. edulis* | 15 | 0.1266 | 0.0697 | 0.0430 | -44.9 | -38.3 |
| | 25 | 0.1266 | 0.0638 | 0.0842 | -49.6 | +31.9 |
| | 35 | 0.1266 | 0.2024 | 0.1322 | +59.9 | -34.2 |
| | 45 | 0.1266 | 0.0927 | 0.1499 | -26.8 | +61.7 |
| *G. crassa* | 15 | 0.0745 | 0.0537 | 0.0511 | -27.9 | - 4.8 |
| | 25 | 0.0745 | 0.0841 | 0.0493 | +13.9 | -41.9 |
| | 35 | 0.0745 | 0.0770 | 0.0879 | + 3.4 | +14.2 |
| | 45 | 0.0745 | 0.0584 | 0.0428 | -21.6 | -26.7 |

**Table 18**

Effect of salinity on phycoerythrin content of Gracilaria spp.

| Species | Treatment | Phycoerythrin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | ppt | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | 15 | 0.4540 | 0.2869 | 0.2483 | -36.8 | -13.5 |
| | 25 | 0.4540 | 0.5034 | 0.2161 | +10.9 | -57.1 |
| | 35 | 0.4540 | 0.3423 | 0.2631 | -24.6 | -23.1 |
| | 45 | 0.4540 | 0.5584 | 0.3932 | +23.0 | -29.6 |
| *G. crassa* | 15 | 0.1681 | 0.1733 | 0.0598 | + 3.1 | -65.5 |
| | 25 | 0.1681 | 0.2990 | 0.0659 | +77.9 | -77.9 |
| | 35 | 0.1681 | 0.3004 | 0.1558 | +78.7 | -48.1 |
| | 45 | 0.1681 | 0.2491 | 0.0700 | +48.2 | -71.9 |

**Table 19**

Effect of salinity of phycocyanin content of *Gracilaria*

| Species | Treatment | Phycocyanin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | ppt | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | 15 | 0.2159 | 0.1325 | 0.0745 | -38.6 | -43.8 |
| | 25 | 0.2159 | 0.2864 | 0.1002 | +32.7 | -65.0 |
| | 35 | 0.2159 | 0.2817 | 0.1145 | +30.5 | -59.4 |
| | 45 | 0.2159 | 0.2874 | 0.1817 | +33.1 | -36.8 |
| *G. crassa* | 15 | 0.1083 | 0.0876 | 0.0196 | -19.1 | -77.6 |
| | 25 | 0.1082 | 0.1955 | 0.0324 | +80.5 | -83.4 |
| | 35 | 0.1083 | 0.1586 | 0.0664 | +46.5 | -58.1 |
| | 45 | 0.1083 | 0.1007 | 0.0254 | - 7.0 | -74.8 |

**Table 20**

Effect of salinity on allophycocyanin content of *Gracilaria* spp.

| Species | Treatment | Allophycocyanin content (mg/g Fw) | | | % of change | |
|---|---|---|---|---|---|---|
| | ppt | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | 15 | 0.1730 | 0.1901 | 0.1642 | + 9.9 | -13.6 |
| | 25 | 0.1730 | 0.2703 | 0.1548 | + 56.2 | -42.7 |
| | 35 | 0.1730 | 0.4006 | 0.1502 | +131.6 | -62.5 |
| | 45 | 0.1730 | 0.1839 | 0.1324 | + 6.3 | -28.0 |
| *G. crassa* | 15 | 0.1083 | 0.1262 | 0.0492 | + 16.5 | -61.0 |
| | 25 | 0.1083 | 0.2464 | 0.0457 | +127.5 | -81.5 |
| | 35 | 0.1083 | 0.2212 | 0.1166 | +104.3 | -47.3 |
| | 45 | 0.1083 | 0.1395 | 0.0404 | + 28.8 | -71.0 |

6.41% in *G. edulis* and 4.09% in *G. crassa* on 6th day of treatments. In 15 ppt the photosynthetic activity declined drastically in both the species of *Gracilaria* on 6th day. At 45 ppt, the decline of photosynthetic activity was marginal initially and more pronounced on the 12 day. In *G. crassa* it declined by 90% of initial activity on 12th day (Table 21).

Room temperature absorption spectra of fresh and treated *G. edulis* and *G. crassa* showed prominent peaks at 676, 621, 565, 495 and 433 nm (Fig. 35). On 6th day of treatment in *G. edulis* the small peak at 541 nm becomes prominent at 25, 35 and 45 ppt but reduced at 15 ppt. The absorption peaks in samples incubated at 35 ppt were better preserved than others when the spectra were normalized at 433 nm (Fig. 36). On 12th day of treatment at 15 ppt, the peaks at 565 and 495 nm were shifted to 563 and 493 nm, respectively. In 35 and 25 ppt, the peaks at 565 and 495 nm were shifted to 560 and 491 nm, respectively. In addition the peak at 541 nm was more prominent on 6th and 12th day of treatment (Fig. 37).

In *G. crassa*, at 45 ppt, the absorption peaks at 676 and 621 nm were prominent on 6th day of treatment but at 495 nm the peak maxima was observed at 35 ppt. In 45 ppt, the peak at 676 nm was shifted to 674, while that of 621 to 624 nm in samples treated with 35 ppt and 15 ppt. The peak at 541 nm was not very prominent (Fig. 38). After 12 days of treatment the peaks at 676, 621, 565, 495 became prominent in 35 ppt. The peak at 676 nm was shifted to 674 in 15 and 25 ppt samples, and to 672 nm in 35 ppt sample. Similarly, a shift of absorption peak at 565 nm to 562 nm for 15 ppt and to 563 nm for 25 ppt was noticed. The peak at 495 nm was found shifted to 497 nm in 15 and 25 ppt but at 35 ppt the peak at 433 nm showed a drastic shift to 441 nm. In samples treated with 45 ppt, the level of major peaks showed drastic reduction (Fig. 39).

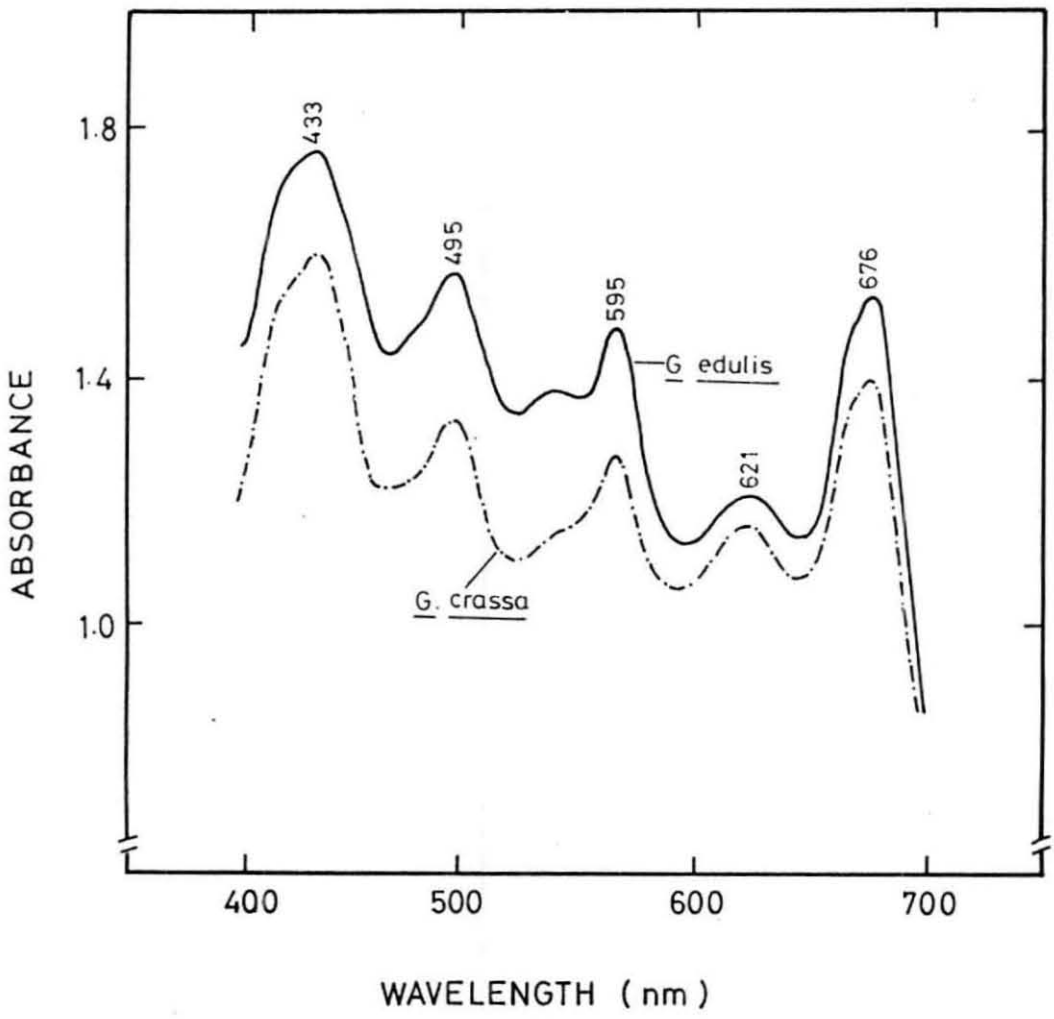


**Fig. 35**

Room temperature absorption spectra of fresh thalli of *Gracilaria edulis* and *G. crassa* collected from Thonithurai. For details of spectral measurements, see Materials and methods.

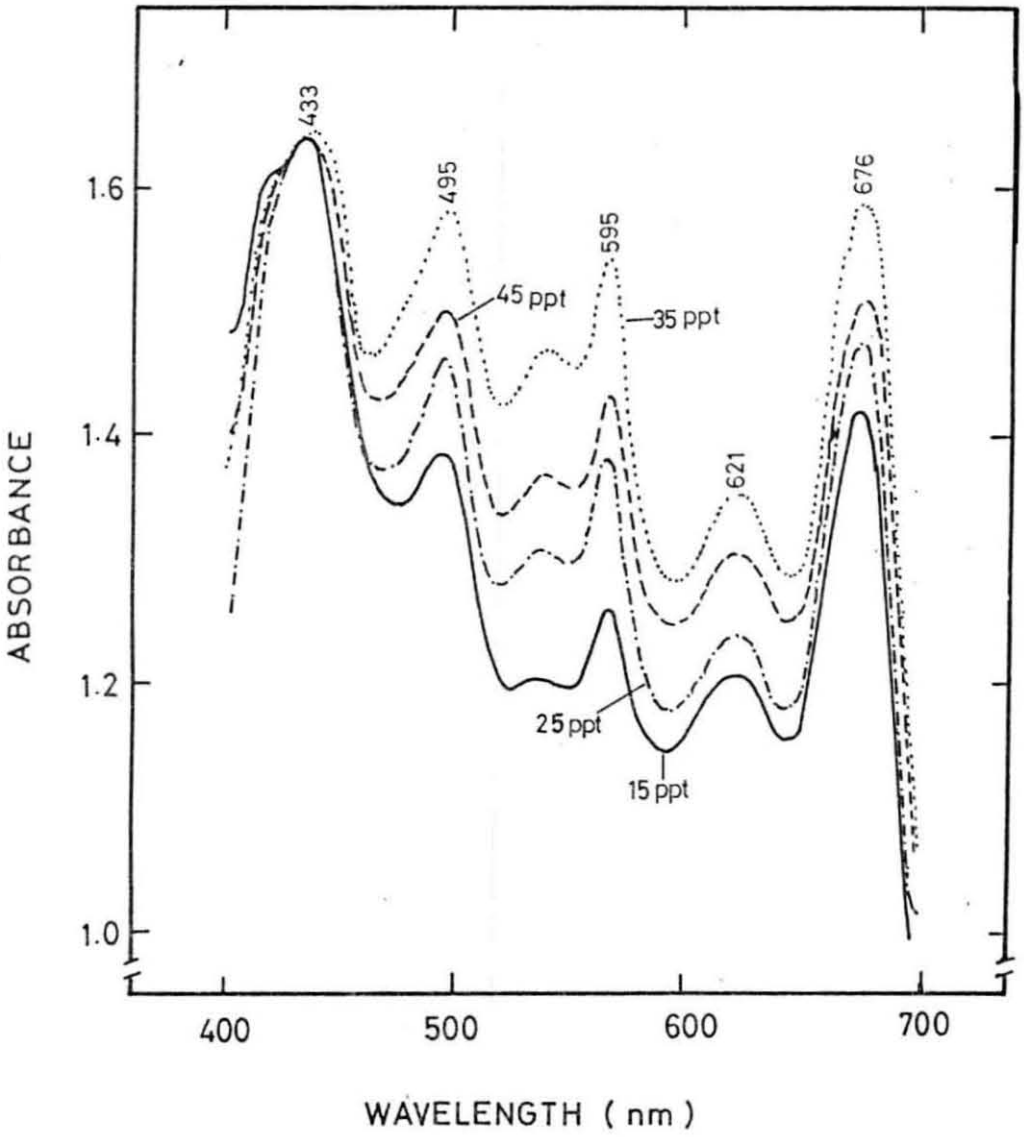


**Fig. 36**

Room temperature absorption spectra of the thallus of *Gracilaria edulis* after 6 days of treatment under different salinities (15, 25, 35 and 45 ppt) maintained in a growth chamber. For details of spectral measurements, see Materials and methods.

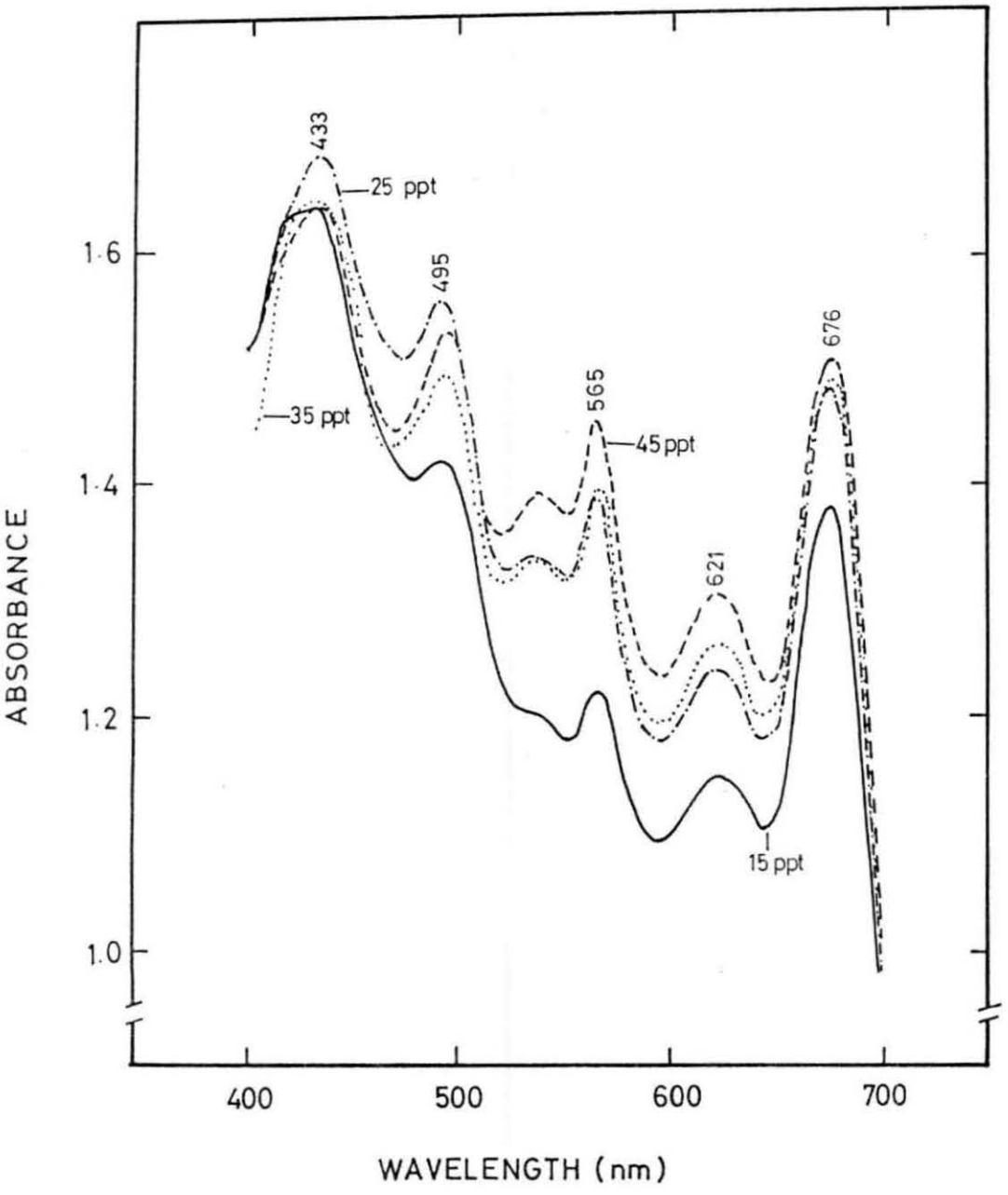


**Fig. 37**

Room temperature absorption spectra of the thallus of *Gracilaria edulis* after 12 days of treatment under different salinities (15, 25, 35 and 45 ppt) maintained in a growth chamber. For details of spectral measurements, see Materials and methods.

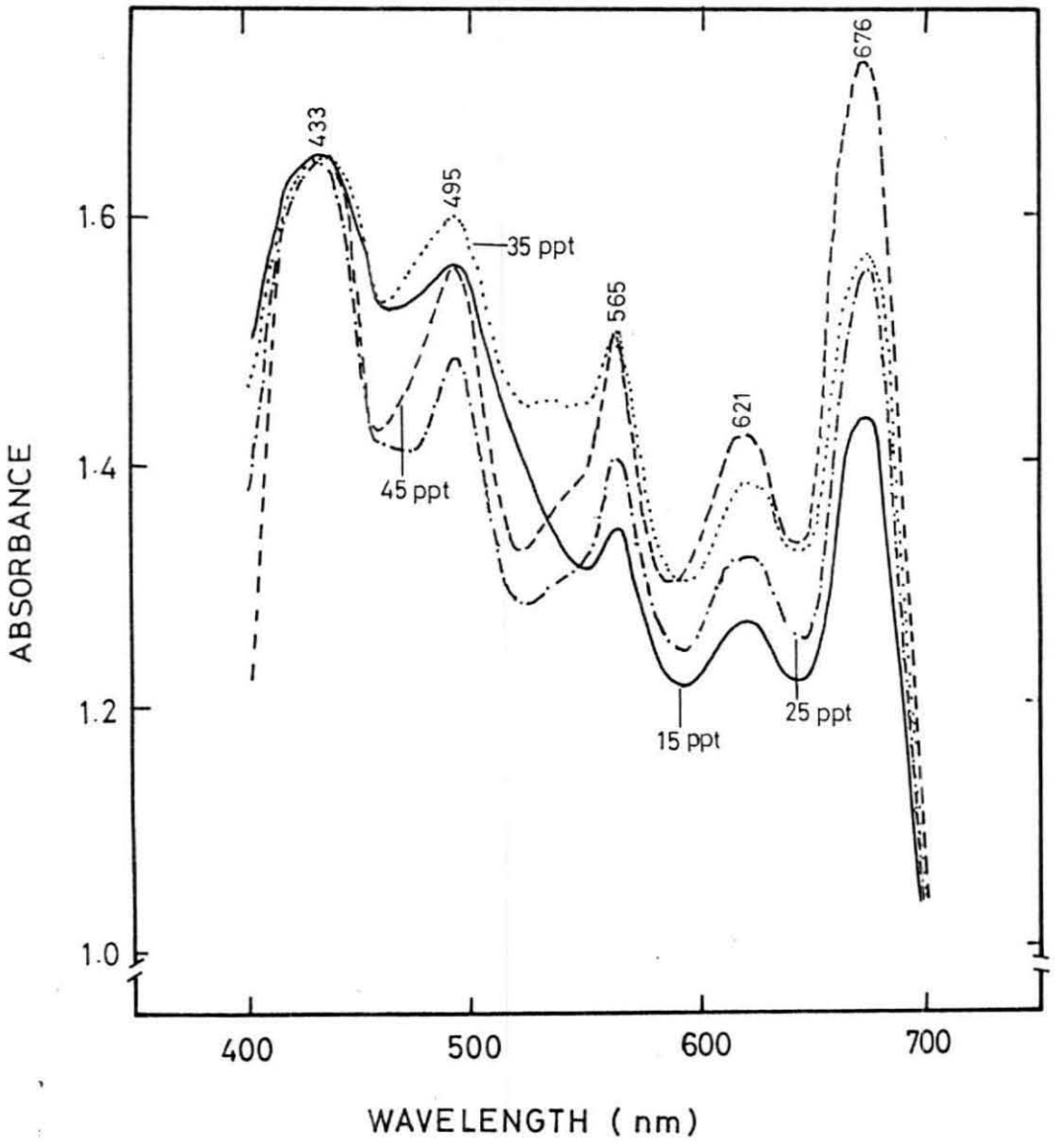


**Fig. 38**

Room temperature absorption spectra of the thallus of *Gracilaria crassa* after 6 days of treatment under different salinities (15, 25, 35 and 45 ppt) maintained in a growth chamber. For details of spectral measurements, see Materials and methods.

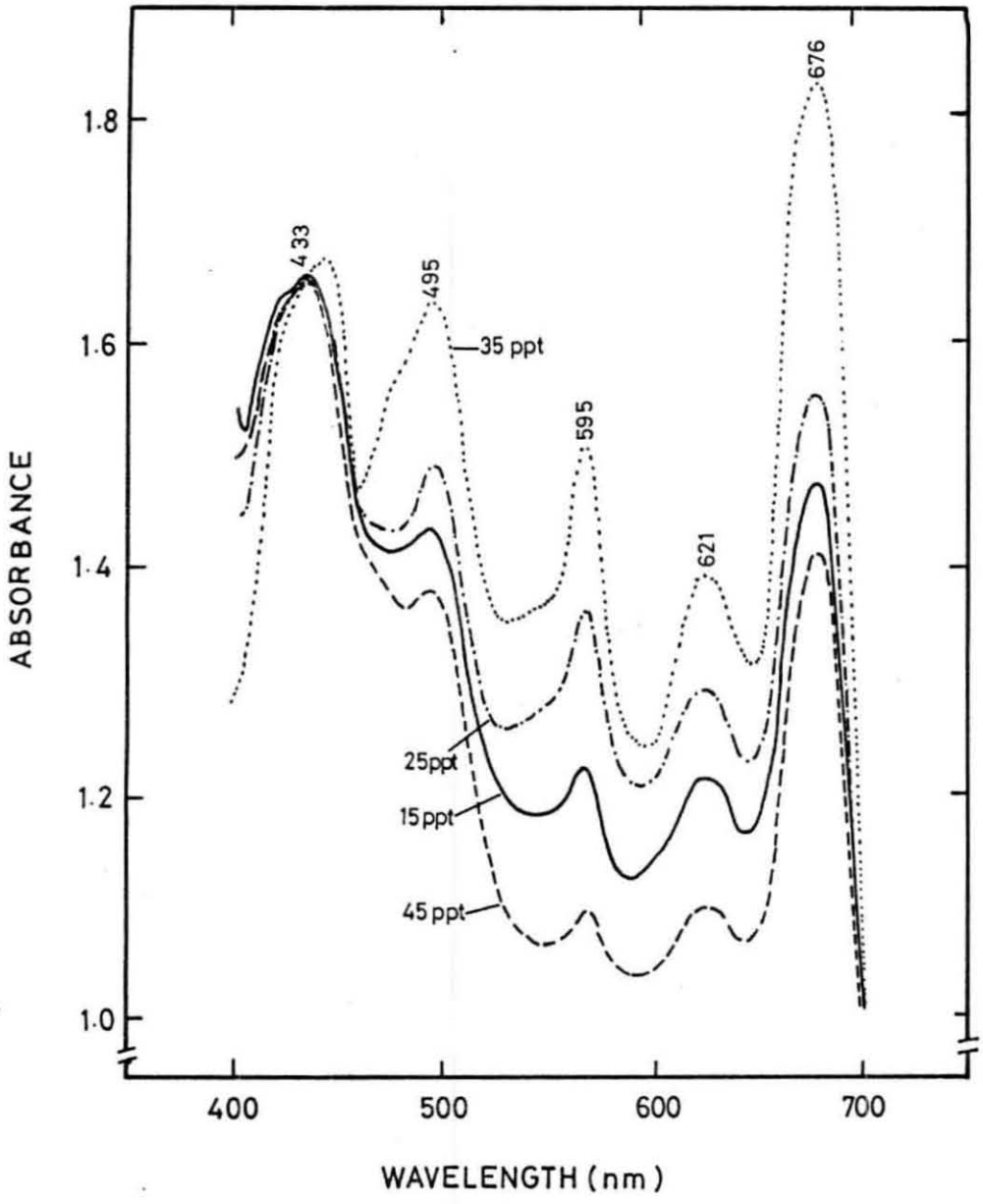


**Fig. 39**

Room temperature absorption spectra of the thallus of *Gracilaria crassa* after 12 days of treatment under different salinities (15, 25, 35 and 45 ppt) maintained in a growth chamber. For details of spectral measurements, see Materials and methods.

**Table 21**

Effect of salinity on photosynthetic activity of Gracilaria spp.

| Species | Treatment | Photosynthetic activity (μM/g Fw/ h) | | | % of change | |
|---|---|---|---|---|---|---|
| | ppt | 0 day | 6 days | 12 days | 0-6 days | 6-12 days |
| *G. edulis* | 15 | 26.54 | 9.12 | 8.00 | -65.3 | -12.3 |
| | 25 | 26.54 | 14.97 | 8.33 | -43.6 | -49.4 |
| | 35 | 26.54 | 24.84 | 9.09 | - 6.4 | -63.4 |
| | 45 | 26.54 | 22.56 | 8.62 | -15.0 | -61.8 |
| *G. crassa* | 15 | 18.33 | 4.55 | 3.38 | -75.2 | -25.7 |
| | 25 | 18.33 | 7.83 | 3.21 | -57.3 | -59.0 |
| | 35 | 18.33 | 17.58 | 7.69 | - 4.1 | -56.3 |
| | 45 | 18.33 | 8.62 | 0.84 | -53.0 | -90.3 |

**Table 22**

Effect of salinities on fluorescence kinetics of Gracilaria spp.

| Species | Treatment | | Fast kinetics | | Slow kinetics | | |
|---|---|---|---|---|---|---|---|
| | | | Variable fluorescence | Quantum yield | Peak value | Terminal value | |
| | days | (ppt) | (Fv) | (Fv/Fm) | (P) | (T) | P-T |
| *G. edulis* | 0 | — | 0.1 | 0.01 | 11.9 | 9.0 | 2.9 |
| | 6 | 15 | — | —— | 11.2 | 8.9 | 2.3 |
| | | 25 | 0.5 | 0.06 | 11.9 | 9.5 | 1.6 |
| | | 35 | 0.3 | 0.04 | 10.4 | 8.5 | 1.9 |
| | | 45 | 2.2 | 0.22 | 13.7 | 11.3 | 2.4 |
| | 12 | 15 | 0.3 | 0.01 | 11.1 | 9.7 | 2.4 |
| | | 25 | 1.4 | 0.04 | 9.9 | 8.4 | 1.5 |
| | | 35 | 1.4 | 0.15 | 8.2 | 6.6 | 1.6 |
| | | 45 | 2.8 | 0.15 | 13.4 | 11.4 | 2.0 |
| *G. crassa* | 0 | — | 3.2 | 0.29 | 19.2 | 11.6 | 7.6 |
| | 6 | 15 | 1.3 | 0.14 | 8.6 | 7.7 | 0.9 |
| | | 25 | 1.6 | 0.17 | 12.0 | 9.0 | 3.0 |
| | | 35 | 2.6 | 0.25 | 10.2 | 8.9 | 1.3 |
| | | 45 | — | —— | 8.0 | 7.3 | 0.7 |
| | 12 | 15 | 0.2 | 0.02 | 13.7 | 11.9 | 1.8 |
| | | 25 | 1.3 | 0.14 | 9.3 | 8.0 | 1.3 |
| | | 35 | 1.5 | 0.16 | 14.2 | 13.3 | 1.3 |
| | | 45 | 1.5 | 0.16 | 8.4 | 7.0 | 1.4 |

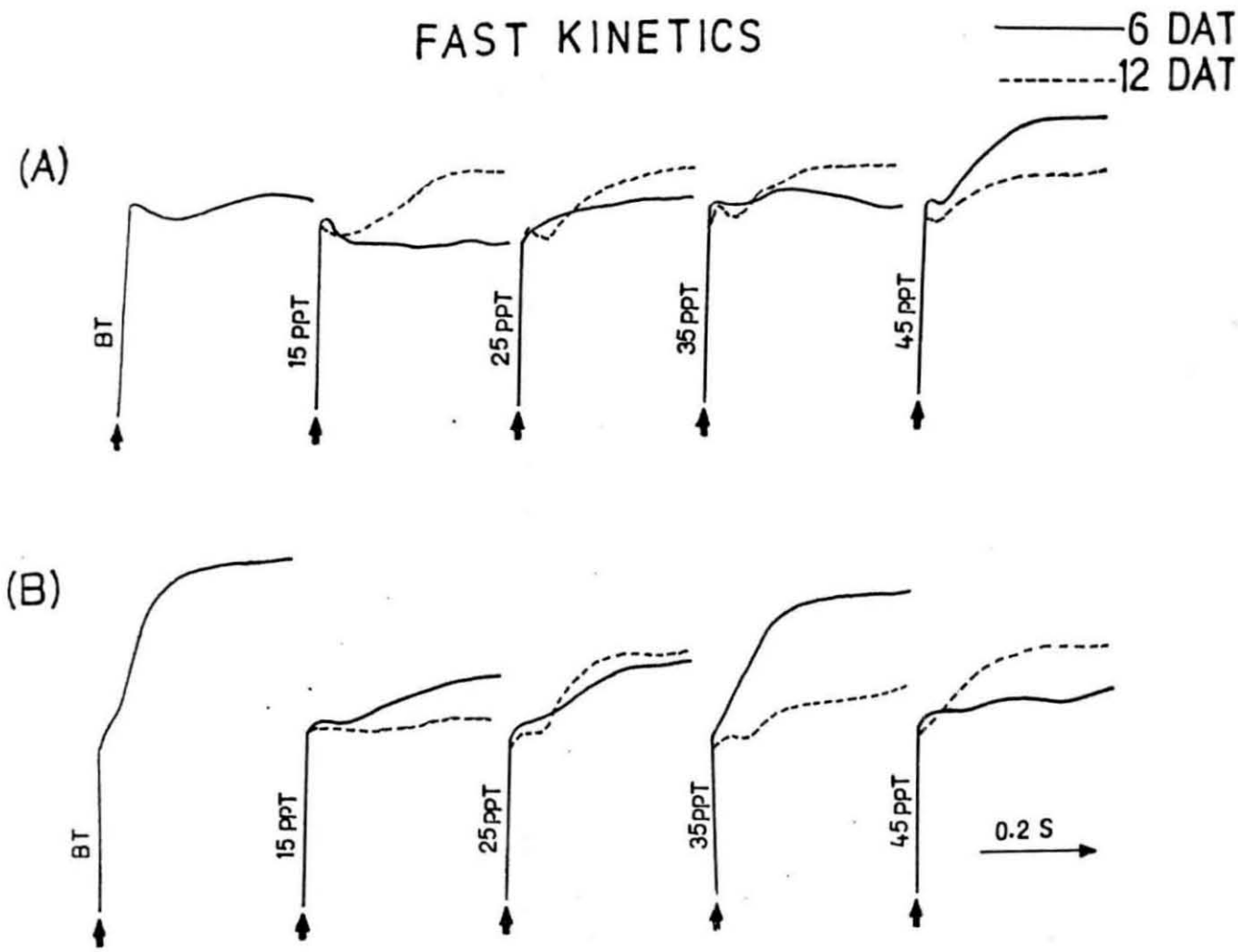


**Fig. 40**

Typical fast fluorescence kinetics of *Gracilaria edulis* (A) and *G. crassa*(B) subjected to treatments under different salinities 6 and 12 days (15, 25, 35 and 45 ppt). For details of fluorescence measurements, see Materials and methods.

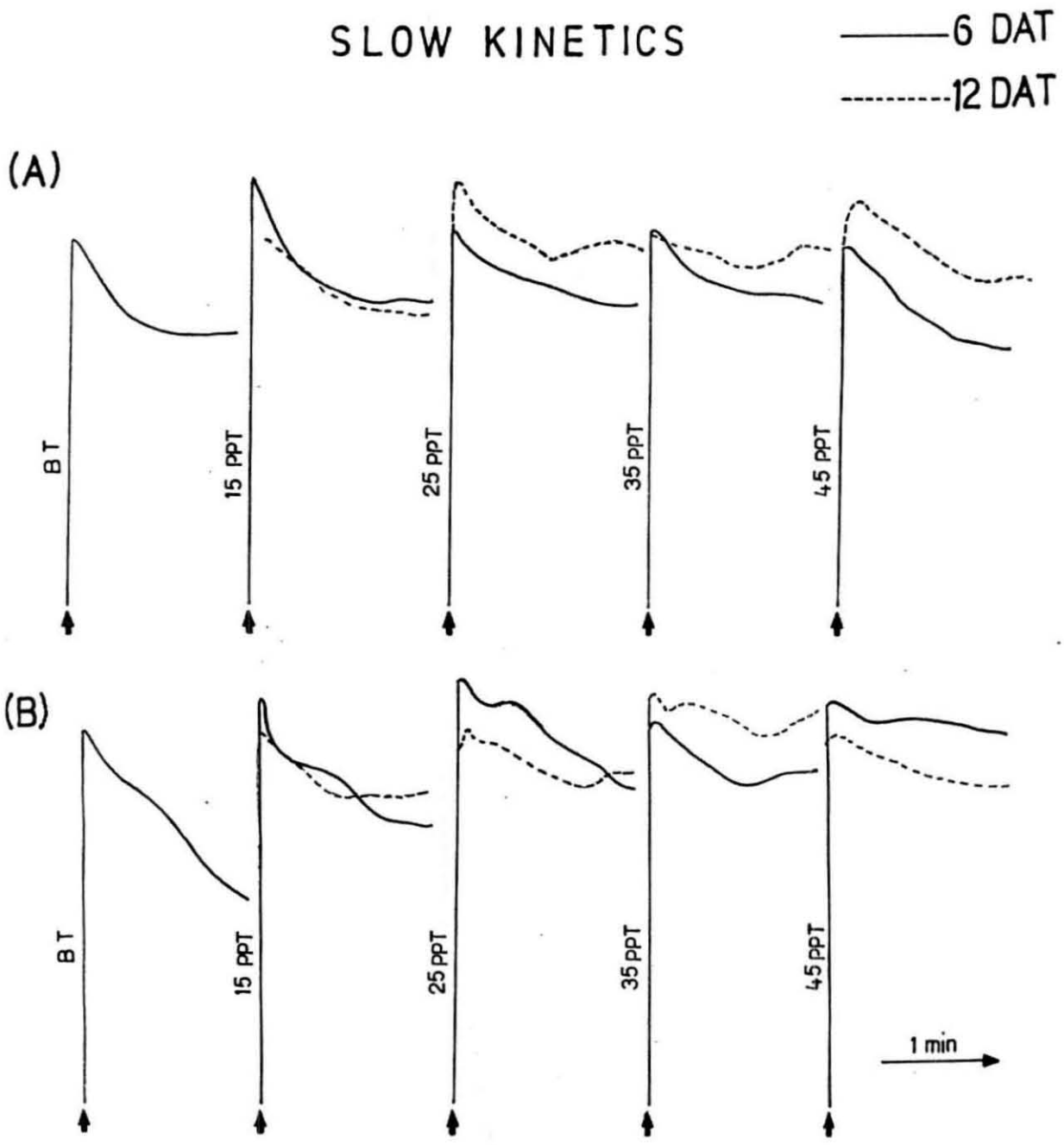


**Fig. 41**

Typical slow fluorescence kinetics of *Gracilaria edulis* (A) and *G. crassa* (B) subjected to treatments under different salinities 6 and 12 days (15, 25, 35 and 45 ppt). For details of fluorescence measurements, see Materials and methods.

**Table 23**

Major fatty acids in the species of *Gracilaria*

| Major fatty acids | Fatty acids (% of total fatty acids) | | |
|---|---|---|---|
| | *G. edulis* | *G. crassa* | *G. corticata* |
| Lauric acid | 1.20 | 0.46 | 0.32 |
| Myristic acid | 22.80 | 18.03 | 20.46 |
| Myristoleic acid | 16.31 | 12.23 | 16.94 |
| Palmatic acid | 12.03 | 8.44 | 11.94 |
| Palmitoleic acid | 11.94 | 11.00 | 16.23 |
| Stearic acid | 1.04 | —— | 1.88 |

**Fluorescence kinetics**

Fluorescence kinetics of *G. edulis* and *G. crassa* showed differences in quantum yield, variable fluorescence, peak and terminal values upon treatment to different salinities (Fig. 40). *G. edulis* exhibited the lowest value of fluorescence induction in control (0 day) sample. At low salinity, the quantum yield was negligible on 6th day of treatment but recovered marginally on 12th day. Maximum quantum yield and Fv values observed at 45 ppt gradually increased till 12th day of incubation. At 25 and 35 ppt, the Fv/Fm value incrased gradually. In *G. crassa* the variable fluorescence and quantum yield were much higher on 0 day and declined in all the treated samples. At 45 ppt, there was a drastic reduction in quantum yield which recovered marginally on the 12th day of treatment. At 35 ppt both Fv and Fv/Fm values were higher than other treated samples (Table 22).

In slow kinetics of *G. edulis* the difference of peak and temrinal value was found to be more on 0 day. Upon treatment, it declined gradually till 12th day. The value was found to be more at 45 ppt. In *G. crassa* the difference of peak and terminal value was too high and declined drastically on treatment to different salinity (Fig. 41). Maximum decline was observed at 15 and 45 ppt which recovered marginally after 12 days of treatment (Table 22).

# Chapter II

# Discussion

Primary productivity is a function of both rate of production and biomass (Dring 1982) for which photosynthesis is the major component. The photosynthetic activity of macroalgae has been shown to be strongly affected by seasonal changes (King and Schramm 1976, Littler *et al.* 1979). Laboratory experiments on photosynthetic activity at different treatments have an additional support to the hypothesis of seasonal pattern (Mathieson and Burns 1971, Brechignac and Andre 1984).

In the present $^{14}C$ incorporation and polarographic studies, the photosynthetic activity was found to be higher in *G. corticata* than in *G. edulis* and *G. crassa*. Maximum photosynthesis was achieved even at 2 $W/m^2$ under laboratory conditions in all the three species of *Gracilaria*. Further, marginal increase of activity was noticed up to 10 $W/m^2$ and declined thereafter. The overall photosynthetic activity was more than double in *G. corticata*. It may be the fact that, in natural habitat, *G. corticata* prefers to grow in the rocky shore of the sea completely exposed to full sunlight during low tide and get immersed in the water only during high tides. Thus, it can utilize maximum light energy compared to the other two submerged species of *Gracilaria*.

The seawater becuase of its high pH, has got ionic forms of carbon in high concentration which can be used by the macrophytes. When exposed to air, these intertidal algae use atmospheric carbon dioxide as their inorganic carbon source which is utilized to form organic compounds. Thus light and the carbon are not the limiting factors for *G. corticata*. Besides, the pigment contents, such as chlorophyll and phycobilins, are more in this species compared to *G. crassa* and *G. edulis*. The morphology of the species could be partly responsible for the observed high photosynthetic activity of *G. corticata*. The thallus is thin and flattened rather than the tubular appearence of *G. crassa* and *G. edulis*.

In marine aquatic system, variations occur in light intensity, light quality and salinity throughout the year. Keeping this in view, some experiments were conducted in the laboratory to study the effect of light intensity, spectral quality and salinity on the various physiological aspects of *Gracilaria*.

**Effect of light intensity**

Light plays a very important role as a factor controlling plant morphology (Dring and Lunning 1983, Dring 1988). About 50% of the incident radiation may be reflected at the surface and less than 20% reaches below a depth of 10 m. Thus the benthic macroalgae show photosynthetic features similar to those found in terrestrial shade plants (Bowes 1985). Low light saturation levels for photosynthesis are characteristic of algae from all the three divisions (Oats and Murray 1983, Reiskind *et al.* 1989). In the present experiment varied response was observed among the species of *Gracilaria* in their photosynthetic activity. *G. edulis* exhibited highest activity under HL intensity whereas in *G. crassa* the activity declined drastically after 6 days of treatment under HL but under LL activity persisted even after 12 days of treatment. *G. corticata* is considered as an actively photosynthezing species, showed enhanced photorespiration after 12 days of treatment. Photorespiration in marine algae has not been well characterized but inhibition of photosynthesis by oxygen is reported in a few macroalgae (Brown *et al.* 1976, Dromogoole 1978a, Reiskind *et al.* 1988).

It is evident at high $O_2$ and low $CO_2$ concentration, developed under the experimental condition in growth chamber using aquarium tanks with stagnant seawater, might have promoted photorespiration. Thus after certain period of active photosynthesis, the dissolved $CO_2$ of seawater reduced with concomittant increase in $O_2$ concentration in the system. In *G. edulis* and *G. crassa* the photosynthetic activity was slow resulting in poor utilization of dissolved carbon and the plant retained photosynthetic activity till 12 days of treatment although at a reduced level.

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Critical light requirements vary from species to species and even in the same species and different habitat. There is an optimum requirement of light for proper growth (Falkowski *et al.* 1985), for saturating photosynthesis (Richardson *et al.* 1982) and sensitivity against photoinhibition (Bose *et al.* 1988, Huppertz *et al.* 1990, Franklin *et al.* 1992). In the present experiment, the critical light intensity required for saturated photosynthesis varied among the species of *Gracilaria*. In *G. corticata*, the light provided in growth chamber was not as high as it received in nature but the saturation has occurred even at low light intensity. Similar observation was made by Oats and Murray (1983) and Reiskind *et al.* (1989). Davis and Dawes (1981) reported that the intertidal species which are exposed to full sunlight, exhibit low light saturation level indicating that this is a general trait among macroalgae regardless of their habitats.

Red algae typically contain Chl *a* and carotene as a major lipophilic pigments along with phycoerythrin, phococyanin and allophycocyanin. The most striking difference between Rhodophyta and other algae is the enoromous variation in their antennae complex. Cyanobacteria and red algae posses large extrinsic phycobilisomes mainly connected to PSII whereas PSI served with small antennae (Morschel and Schartz 1988).

Chlorophyll *a* is the major pigment and is present in all photosynthetic organisms that evolve $O_2$. Increase of pigment content at low light intensity is of general occurrence but changs in chlorophyll content are species specific. *G. edulis* showed higher chlorophyll content at 3 $W/m^2$ whereas in *G. crassa*, chlorophyll contnet was higher only at low light intensity (0.5 $W/m^2$). In *G. corticata*, prolonged treatment to these light intensities reduced the pigment content of all kinds. It may be explained that after a brief period of active photosynthesis *G. corticata* exhibited photorespiration which results a change of the pH of stagnant seawater. This probably might have led to the loss of pigments and finally to bleaching of the plants.

The rate of primary production is determined by quantifying PSII activity and by linking its activity to whole carbon fixation (Kroon *et al.* 1993). The two most commonly employed techniques to estimate PSII quantum yield are measurement of chlorophyll fluorescence and oxygen evolution. The time course of fluorescence induction kinetics from L adapted *G. edulis* samples show higher values of variable fluorescence and quantum yield. Cyanobacteria and red algae are known to transfer energy from phycobilisomes to PSII. The large antennae system implies consequences for the fluorescence parameters which often show very high value in relation to Fm leading to decreased Fv/Fm ratio in red algae compared to higher plant (Hauelt *et al.* 1992). The peak and the terminal values of slow kinetics are due to the transient imbalance of electrons consumed by the enzymatic dark reactions and the electron delivered by the light reactions (Seaton and Walker 1990). The strong reduction from P to S mainly by the formation of the pH gradient across the thylakoid membrane which is not very prominent in marine algae. The peak and the terminal levels of *G. edulis* were also found to be higher under IL. Thus, there is a proportional relationship between PSII activity and carbon dioxide fixation in *G. edulis* under IL intensity. Similar results were found in *G. crassa* under LL intensity. In *G. corticata* the quantum yield and variable fluorescence did not show much variations at different light intensities but the peak and the terminal levels exhibited wide variations which indicates the occurrence of some activities other than carbon dioxide fixation.

Despite the plentiful diversity of macroalgae in coastal environments, a few studies have examined *in vivo* absorption features of common macrophytes. In the present study, the thallus absorbance spectrum under different light treatments showed peaks at 676, 621, 565, 495 and 433 nm representing Chl *a* and accessory pigments such as PE, PC, APC and carotenoid, respectively. PE has a characteristic absorption band of at 498 nm attributable to the presence

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of phycourobilins chromophores (Kursar and Alberte 1983). The shoulder near 433 nm under LL conditions in *G. edulis* is attributable to a change in chlorophyll content and composition as observed in *Mastocarpus papillatus* (Owen *et al.* 1987). The absorption at 627-630 nm is attributable to PC (Kursar and Alberte 1983). In general, the critical light required for the active photosynthetic metabolism for *G. edulis* may be within 2 $W/m^2$ where as in *G. crassa* lower light intensity is favoured. *G. corticata* may require continuous replenishment of $CO_2$ in the system for active photosynthetic activity.

**Effect of light quality**

The theory of complementary chromatic adaptation suggests that red seaweeds are best adapted for life in the deep sea because of inclusion of PE in the photosynthetic unit. The mechanism involved in this process is well studied in Cyanobacteria. However, there is still controversy about the occurrence of complementary chromatic adaptation in red algae.

In the present study, all the three species exhibited different activities under spectral influence. The photosynthetic activities of *G. edulis* and *G. corticata* were influenced by blue light in the initital stage but prolonged treatment retarded the activity. *G. edulis* and *G. corticata* retained the ability to maintain photosynthetic activity under red light. The growth of the thallus was also more prominent under this treatement. Plants were highly elongated and branched. Similar results were reported in other red algae such as *Porphyra*, *Palmaria palmata* (Luning 1992) and *Gracilaria* (Beer and Levy 1983). It was suggested by earlier workers that red light favoured thallus expansion, cell division, carbon accumulation and its deposition in cell walls and intercellular matrix. Figueroa (1995) has opined that thalli grown under red light showed smaller protoplasmic area and ample intercellular matrix which are morphological indication that polysacchride production is favoured. Figueroa (1994) explained that oxygen production was three times higher in algae growing under red

light than under blue light. Similar observations were made in the present study. *G. corticata* which exhibited photorespiratory activity in all the spectral range show photosynthetic activity under RL even after 12 days of treatment. No adverse effects of long time cultivations in red light were observed for *Porphyra umbilicalis*, which indicates that this spectral range serves both photosynthetic and morphogenetic demand of growth. In *G. crassa* the photosynthetic activity was less in all treated samples including under RL. It may be due to the fleshy and rigid thallus structure of *G. crassa*.

Chlorophyll contents of *G. eulis*, *G. crassa* and *G. corticata* were found to be higher in all the light treatments for 6 days. Prolonged treatment retarded the pigment content in BL, WL and GL but RL enhanced the pigment content. Figueroa and Niell (1989) demonstrated the involvement of phytochrome in the control of chlorophyll synthesis in red algae, *Porphyra umbilicalis*. Like other algae, BL also helps in enhancement of chlorophyll content in *G. edulis, G. corticata* and *G. crassa*. In green algae chlorophyll synthesis is regulated by specific BL photoreceptors (Senger and Baker 1987).

The accessory pigments were found to be higher under GL in all the species of *Gracilaria* after 6 days of treatment but prolonged treatement exhibited reduction of all the accessory pigments in *G. corticata*, but increased in red, blue and green light in *G. edulis* and *G. crassa*.

The observed better growth and photosynthesis under RL than BL may be due to the higher quantum yield in the former. The spectral range for BL used may not be optimal for the photosynthesis because only the PSI was well supplied with photons while PSII was inadequately served. Increase in chlorophyll content under red light might induced PSII activity and carbon fixation although the accessory pigments are comparatively less. Continued photosynthetic activity in *G. corticata* under RL may support the hypothesis that chloroplast activity and long wavelength light are critical for pigment biosynthesis in *G. corticata*.

The absorption peaks of the spectra of plants maintained under different light quality showed shift of peaks at 565, 621 and 676 nm which are attributable to the change of accessory pigments. In all the species of *Gracilaria*, it was obsersved that the peaks under red light were more in height. *G. crassa* and *G. corticata* showed prominent shoulders at 450 nm and several peaks and troughs between 433 and 450 nm which may be attributed to changes in the composition of chlorophyll as observed by Smith and Alberte (1994). The absorbance change at 515 nm has been attributed to a number of components (Chlorophyll and carotenoids) functioning in the electron transport chain near PSII. This may partly indicate a change in membrane potential. Thus the change at 515 nm presumably shows participation of carotenoids in photosynthesis of algae.

**Effect of Salinity**

Salinity is another important parameter which influences the physiological status of marine algae. Due to the sensitivity to low light intensity under laboratory condition, *G. corticata* was not employed in this experiment.

The photosynthetic activity of *G. edulis* and *G. crassa* declined gradually from 0-12 days of treatment under different levels of salinities. The decline in photosynthetic activity as a function of time was evidence for all the experiments conducted indicating that such changes may be due to gradual decline of dissolved carbon content in the stagnant seawater. Drastic decrease in activity was noticed beyond 6 days of treatment. Among the different concentrations tried, high photosynthetic activity was noticed under 35 ppt in *G. edulis* followed by 45 ppt. Although prolonged incubation retarded the photosynthetic activity drastically in all the treated samples, those kept in 35 ppt maintained higher activity.

Fluorescence kinetics studies, indicated high quantum yield, initial peak and steady state level only in samples maintained at optimum salinity which

confirms the efficient functioning of PSII and carbon fixation reactions. The fluorescence transients were better in *G. crassa* then in *G. edulis*. Similar results were also found on slow fluorescence kinetics.

Salinity changes the turgor of newly formed thalli. In *Porphyra purpurea* turgor increases with decresing salinity (Reed *et al.* 1980a). Similar observations were also made in the present study. The water retaining capacity increases as salinity declines in *G. edulis* and *G. crassa*. In some red algae first, second and third orders of branches were observed (Jordan and Vadas 1972). A decrease in salinity may result in short term lower photosynthetic rate and higher $Na^+$ - $K^+$ pump, which is believed to the major mechanism algae (Gessner and Schramm 1971). In *Griffthsia monilis* and other species, it was also noticed that turgor in maintained by the activity via ion pumping and perhaps by synthesis of digenaside (Bisson and Kirst 1979). Reed *et al.* (1980b) showed an increase in floridoside level with increase in salinity in *Porphyra purpurea*. In the present study, low photosynthetic activity was evident under low salinity. This is in conformity with several earlier studies (Yarish *et al.* 1979, Coudret *et al.* 1983). There is also a report indicating that photosynthetic and respiratory rates declined at higher salinity in *Gelidium monilis* (Kirst 1981) Macler (1988) suggested that *G. coulteri* can grow even at 50% salinity but with net surplus of photosynthesis due to inhibition of carbon fixation and increase in photorespiration.

Absorption spectra of thallus of *G. edulis* and *G. crassa* showed variations under treatment of different salinities. The prominent peaks at 676, 621, 565, 495 and 433 nm are attributed to the major photosynthetic pigments. In *G. edulis*, the major peaks at 565 and 495 nm were shifted to a certain extent could be attributed to changes in the chlorophyll and phycobilins.

# Summary

*Gracilaria* is found seldom in extreme exposure. More commonly, the plants are attached to shells, small stones, pebbles or small objects. More than 130 species of *Gracilaria* present worldwide from which 100 or so are agarophytes. In India, thirteen species are recorded from different coast. The main natural agarophyte resources of India consist of different species of *Gracilaria* and *Gelidiella* found in the South-east coast of Mandapam and Tuticorin area of Gulf of Mannar, which is found to be the nodal region for commercial exploitation of economic seaweeds.

Increasing demand of agrophytes coupled with depletion of natural stocks necessitates propagation of some species of *Gracilaria* in large scale. Species of *Gracilaria* such as *G. edulis*, *G. crassa* and *G. corticata* exhibit a wide variation in their morphology, habitat and other physiological parameters such as biomass, pigment consitituents, photosynthetic efficiency, respiratory activity, agar yield and quality, biochemical constitutents and mineral constitutents.

*G. edulis* is cylindrical and regular dichotomously branched found in shallow area of sea protected by islands. *G. crassa* formed a dense cushion on the substration in the similar habitat. The plants are more fleshy rigid and small. *G. corticata* is thick, flattened and dichotomously branched found attached to the rocks or basal part buried under sand. The plants prefer to grow near severe wave action.

Bimodal growth pattern is a general trend in all the species of *Gracilaria* although the peak period of growth varied from spsecies to species. The growth rate was found to be minimum during the month of May. Dry weight varied from 10 to 20% of the fresh weight. Which has got a reciprocal relationship with biomass. In *G. edulis* and *G. corticata* the chlorophyll content was found to be high during peak period of growth where as accessory pigments dominate during lean period of growth. In *G. crassa* the variation in chlorophyll and accessory pigments showed a similar trend. Seaweeds maximize their photosynthetic capacity by optimizing pigment levels. Field photosynthetic

activity was found to be high in *G. edulis* and correlates with peak growth period. The respiratory activity do not show any definite trend but it is found to be maximum during May. The ratio between net photosynthesis and net respiration show gradual increase from September to January and then declined till May. It further increased during July corresponding to the growth rate. The agar content of *Gracilaria* is negatively correlated with grwoth. In general the quality of agar declined in mature tissue when yield in high. The quality of agar is also dependant on other environmental factors. Biochemical constitutents such as protein, carbohydrate and lipid showed almost a reciprocal relationship with growth. Mineral constitutents such as Zinc and Manganese were found to be more during peak period of growth. The constitutents are found in an order such as Na> Ca> Mg> Fe> Zn> PB> Mn> Cu.

The shallow water habitat of *Gracilaria* species is subjected to seasonal variations in temperature, light intensities, light qualities and salinities. Low light saturation is a general trend in all the species of *Gracilaria*. Photosynthetic activity under room temperature showed higher activity in *G. corticata* than those of *G. crassa* and *G. edulis*.

Prolonged period of treatment with different light intensities and qualities under laboratory condition retard the photosynthetic activity drastically and exhibit photorespiration which may be due to depletion of dissolved $CO_2$ and increase in oxygen concentration in the medium. The pigment content declined to a marked extent after 6 days of treatment. Under intermediate light of 2 W/m$^2$ *G. edulis* maintained high quantum yield and variable fluorescence transients. Similar observation was observed in *G. crassa* under low light intensity (0.5 W/m$^2$). Increase in photosynthetic activity and chlorophyll content was observesd in species of *Gracilaria* which are maintained under red light. Accessory pigments were found to be more under green light. A salinity optimum of 35 ppt was found necessary to maintain high photosynthetic activities of *G. edulis* and *G. crassa*.

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